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NOTICE TO OUR READERS

With this issue of the ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA, a change in printers has been made. During the entire 53 years it has been published, the ANNALS has been printed by the same firm, The Spahr & Glenn Company of Columbus, Ohio, and it was, therefore, with considerable reluctance that a change was made. The contract for printing the ANNALS in 1961 was awarded to The Horn-Shafer Company of Baltimore, Maryland, the same firm which now prints the BULLETIN and the MISCELLANEOUS PUBLICATIONS of this Society.

This change was made for reasons of economy and convenience. Use of the same size of type as now used in the JOURNAL OF ECONOMIC ENTOMOLOGY has been adopted, resulting in approximately 10% more words per page where the 9-point type is used. Reprints will be supplied much more promptly than in the past. A more attractive cover has been adopted. We hope that our readers will approve of all of these changes. Your suggestions for continued improvement in the ANNALS are solicited.

F.W.P.

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A price schedule for reprints of papers from the periodicals of the Entomological Society of America was adopted by the Governing Board at the 1955 meeting in Cincinnati. The schedule was printed on page 35 of the December 1955 BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA. This schedule has remained in effect until this writing in spite of the fact that the cost of the reprints to the Society was increased in 1957 and again in 1960. The Governing Board at the 1960 meeting in Atlantic City carefully considered the matter and voted to increase our prices. We hope it will be possible to maintain this new schedule as well as we did the previous one.

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Annals of
The Entomological Society of America

Volume 54 JANUARY, 1961 No. 1

Descriptions of Four New Species of *Culex* from Panama (Diptera: Culicidae)[1]

PEDRO GALINDO[2] AND EUSTORGIO MENDEZ[2]

ABSTRACT

Culex (*Melanoconion*) **johnsoni,** *C.* (*M.*) **keenani,** and *C.* (*M.*) **mesodenticuatus** are described from males. The first-named was taken by sweeping, the others at light. *Culex* (*Mochlostyrax*) **arboricolus** is the second described species of its subgenus whose larva has the scales of the eighth segment arranged in a patch instead of in a single row, and is the second *Mochlostyrax* known to breed in tree-holes; its male, larva, and pupa are described and figured.

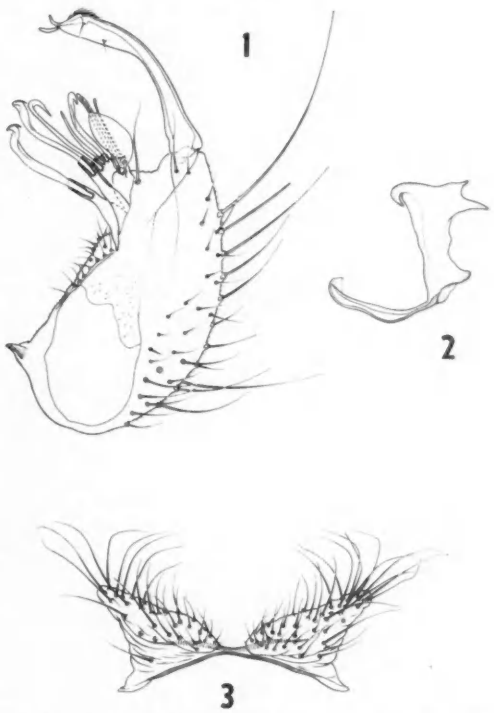
Culex (Melanoconion) johnsoni Galindo and Mendez, n. sp.

MALE.—Unknown, except for terminalia.

Male Terminalia.—Basistyle conical, about twice as long as its greatest width, bearing the usual deciduous stiff hairs and two prominent setae, one inserted at base of outer division of subapical lobe, the other near insertion of dististyle. Outer division of subapical lobe broadly columnar, bearing a large, clear leaf inserted on a prominent tubercle and the following appendages, starting at the inner corner: a hook-tipped filament; a short, slender, rodlike appendage; a large, bent, broad spine with recurved tip, and three closely appressed filaments with expanded apices. Inner division of subapical lobe with arms about equal in size and widely divaricate from base, appendages subequal in size, sinuate, each ending in a recurved spine. Inner plate of phallosome erect, with three points as follows: a laterally curving horn located at apex of ventral border; a short, pointed tooth inserted on dorsoapical border opposite this horn; and a long, sharp spine more basad on dorsal border. Lobes of ninth tergite conical, with the tip of the cone pointing outward, bearing stiff hairs on its surface which grow progressively longer from base to apex.

Type Material.—Holotype: male terminalia mounted on a slide, from a male collected by sweeping at Pacora, Panama, October 1958. Type to be deposited at the U. S. National Museum.

This species can be separated from all other *Melanoconion* by the shape of the inner plate of the phallosome and of the lobes of the ninth segment. We take pleasure in naming it after Dr. Carl M. Johnson, Director of Gorgas Memorial Laboratory.



Culex (*Melanoconion*) *johnsoni,* new species. FIG. 1.—Style. FIG. 2.—Phallosome. FIG. 3.—Lobes of the ninth tergite.

[1] Partial cost of publication of this paper was met by Gorgas Memorial Laboratory. Accepted for publication September 22, 1960.

[2] Gorgas Memorial Laboratory, Apartado 6991, Panama, Republic of Panama.

Culex (Melanoconion) keenani Galindo and Mendez, n. sp.

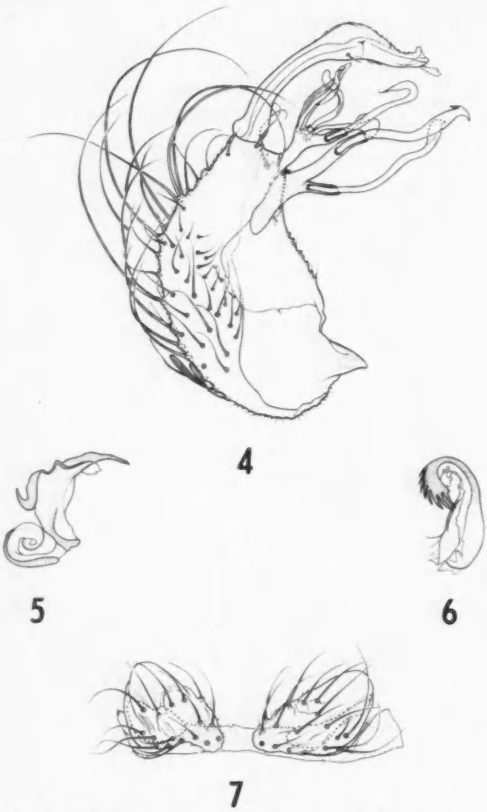
MALE.—A small, light-colored, unmarked *Melanoconion*.

Male Terminalia.—Basistyle slightly more than twice as long as its greatest width, clothed with the usual stiff, deciduous hairs and two prominent setae, one at base of outer division of subapical lobe, the other near insertion of dististyle. Inner division of subapical lobe split into two widely divaricate, unequal arms; lower arm much shorter than upper one, bearing a rather slender appendage ending in a capitate tip, appendage of upper arm similar in shape but shorter and thicker. Outer division narrowly columnar, expanding apically into two arms, inner arm bearing the usual hook-tipped filament accompanied by a much smaller, rodlike appendage, outer arm with three or four closely appressed filaments; inserted between the two arms there are two appendages, an inner one which is slender for most of its length and expands apically into a spatulate tip, and an outer filament bent beyond the middle. Dististyle slightly less than half as long as basistyle, narrowed at middle, then expanding abruptly on apical third and finally tapering into a truncate, upturned, snout-shaped tip. Inner plate of phallosome erect; dorsal corner produced into a long, narrow point, ventral corner bearing a heavily sclerotized, recurved horn. Lobes of ninth tergite rather small, smooth and hairless apically, and with a basal protuberance clothed with several rows of short, stiff hairs.

Type Material.—Holotype male with terminalia mounted on a slide, collected at light in a Shannon trap at Pacora, Panama, on May 27, 1959. Paratype male with same data as holotype. Holotype to be deposited in the U. S. National Museum; paratype in the Gorgas Memorial Laboratory collection.

This species keys out in Rozeboom and Komp (1950) to *conspirator*; it is easily separated by the lack of a ribbed leaf on the outer division of the subapical lobe and by the shape of the inner plate of the phallosome and of the lobes of the ninth tergite.

We take pleasure in naming this species after Mr. Marvin Keenan, of the Malaria Control Force, USARCARIB, whose enthusiastic and unceasing collecting efforts during the past 10 years have contributed greatly to our knowledge of the insects and mammals of Panama.



Culex (*Melanoconion*) *keenani*, new species. FIG. 4.—Style. FIG. 5.—Phallosome. FIG. 6.—Tenth sternite. FIG. 7.—Lobes of ninth tergite.

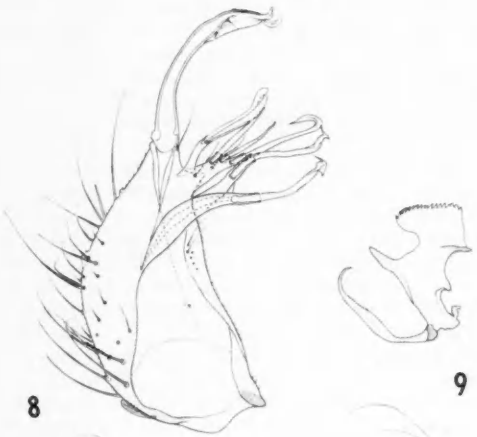
Culex (Melanoconion) mesodenticulatus Galindo and Mendez, n. sp.

MALE.—A small, unmarked *Melanoconion*.

Male Terminalia.—Basistyle slightly more than twice as long as its greatest width, with the usual long, stiff, deciduous hairs along its dorsal margin. Inner division of subapical lobe with two subequal, widely divaricate arms; appendage of inner arm straight for slightly more than half its length, then bending outwardly toward apex and ending in a capitate tip; appendage of outer arm similar in shape, but sinuate from its base. Outer division of subapical lobe divided; inner arm broad, bearing a hook-tipped appendage, a rodlike filament, a flame-shaped filament, and a short, slender seta; outer arm columnar in shape, bearing apically three closely appressed filaments. Dististyle narrowly snout-shaped, with a short, broad, subapical, tonguelike appendage. Lateral plate of phallosome erect, apex broad and bearing a double row of closely appressed, short denticles; dorsal border with a short, heavily sclerotized tooth; ventral border with a long, straight, heavy horn inserted at about the middle. Lobes of ninth tergite ovoid, covered with stiff hairs which grow progressively longer from base to apex.

Type Material.—Holotype: male, mounted on a slide, with terminalia dissected, stained, and mounted separately on same slide. Specimen taken at light in the vicinity of Almirante, Bocas del Toro Province, Republic of Panama, on April 17, 1953. Type to be deposited at the U. S. National Museum.

This species keys out in Rozeboom and Komp (1950) to *C. albinensis*, from which it may be sepa-



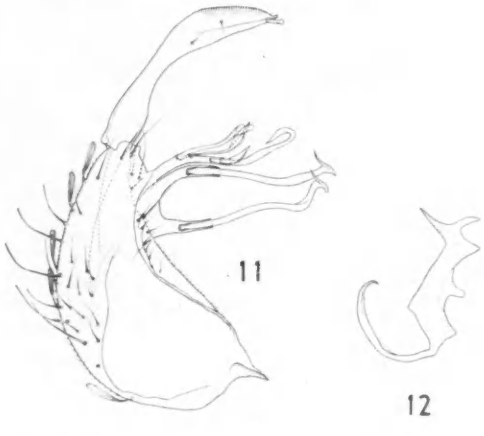
Culex (*Melanoconion*) *mesodenticulatus*, new species. FIG. 8.—Style. FIG. 9.—Phallosome. FIG. 10.—Lobes of ninth tergite.

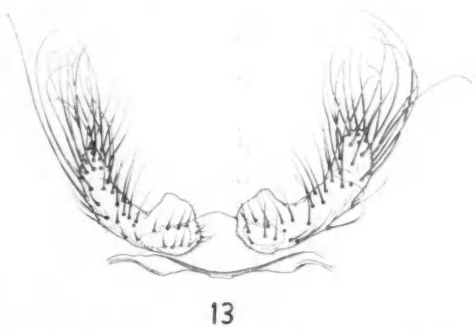
rated by the long columnar arm on the outer division of the subapical lobe (which is absent in *albinensis*), as well as by the shape of the lobes of the ninth tergite.

Culex (Mochlostyrax) arboricolus Galindo and Mendez, n. sp.

MALE.—A small, unmarked *Mochlostyrax*.

Male Terminalia.—Basistyle conical, over twice as long as its greatest width, bearing the usual stiff, deciduous hairs and scales along dorsal surface, and two conspicuous setae on ventral border, one near base of outer division of subapical lobe and one near insertion of dististyle. Inner division of subapical lobe with widely divaricate arms, each with a long appendage which ends in a capitate tip; outer division of subapical lobe narrowly columnar, inner corner produced apically, bearing a long, recurved spine and (usually) an additional short, broad, tonguelike process, outer corner with a cluster of some four closely appressed, filamentous appendages. Dististyle more or less foot-shaped in outline, narrowed at about the middle, then expanded apically. Lateral plate of



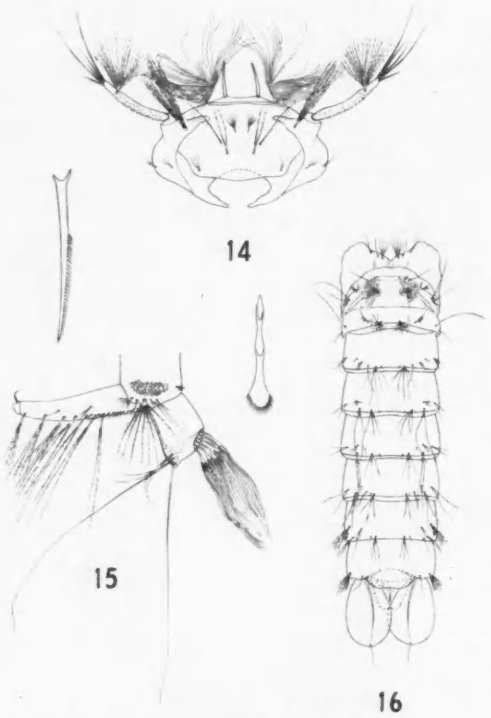


Culex (*Mochlostyrax*) *arboricolus*, new species. FIG. 11.—Style. FIG. 12.—Phallosome. FIG. 13.—Lobes of ninth tergite.

phallosome erect, apex with a short, recurved spine pointing ventrally and a long, slender one directed dorsally; ventral border produced subapically into a short, broad, triangular point. Lobes of ninth tergite rather long and slenderly saclike, with a naked basal protuberance, rest of the lobes mostly covered with long hairs which increase progressively in length from base to apex.

LARVA.—Head wider than long, bulging at the sides. Dorsal head hairs as follows: 4 a small, multiple tuft, 5 double, 6 single, 7 a long tuft with six to eight branches, 8, 9, and 10 very small, double or triple. Antenna spinose, with a long, multiple, subapical tuft. Lateral scales of eighth segment in a broad patch, the individual scales spatulate with spinules at apex. Air-tube about 4x1, tapering gradually from base to apex, tip slightly upturned; pecten teeth on basal third; four to six pairs of long, two- to four-haired, ventral tufts which gradually decrease in size from base to apex; basal tuft within the pecten; a pair of one- to two-haired subdorsal tufts. Anal saddle ringing the segment. Hair 2 a small two- to three-haired tuft, hair 3 single and very long.

PUPA.—Paddles ovoid, midridge well marked to



Culex (Mochlostyrax) arboricolus, new species. FIG. 14.—Head of larva. FIG. 15.—Terminal abdominal structures of larva. FIG. 16.—Pupa.

apex, apical hair short and single. Chaetotaxy as figured.

Type Material.—All from Cerro La Victoria, Panama. *Holotype*: pinned male, collection number 01306, with terminalia mounted on slide, January 4, 1950. *Allotype*: pinned female, collection number 00356, with associated larval and pupal skins mounted on slide, May 18, 1949. *Paratypes*: three males with terminalia, three larval, and four pupal skins mounted on slides, same data as holotype; two males with terminalia, two larval, and two pupal skins mounted on slides, August 11, 1949; one male with terminalia mounted on slide, January 26, 1950; three males and one female, pinned, two larval and one pupal skins mounted on slides, same data as allotype; one male terminalia, three larval, and three pupal skins mounted on slides, December 21, 1949; one male terminalia mounted on slide, September 8, 1951. Types are to be deposited at the U. S. National Museum and at the Gorgas Memorial Laboratory collection.

Discussion.—This new species falls close to *C. caudelli* D. and K. and to *C. vexillifer* Komp. It may be separated from the former as follows: (1) In *caudelli* the terminal spines of the phallosome point almost straight outward, thus producing a deep groove between them, while in *arboricolus* they are directed ventrally and dorsally, respectively, so that no groove is formed between them; (2) The lobes of the ninth tergite in *caudelli* bear an acutely triangular basal protuberance, while in *arboricolus* this protuberance is low, rounded, and not nearly as prominent; (3) The apices of the lobes of the ninth tergite are widely expanded and club-shaped in *caudelli*, while in *arboricolus* these lobes are less widely expanded so that they do not appear club-shaped in outline; (4) In *caudelli* the larva has the scales of the eighth segment in a single row, whereas in *arboricolus* these scales are arranged in a large patch. *C. caudelli* is a ground-pool breeder, while *arboricolus* breeds in tree-holes.

From *C. vexillifer*, whose larva also occurs in tree-holes and bears the scales of the eighth segment in a patch, *C. arboricolus* may easily be separated by the shape of the terminal spines of the phallosome, which are very short in *vexillifer* and long in *arboricolus*, as well as by the shape of the lobes of the ninth tergite.

C. arboricolus is the second described *Mochlostyrax* whose larva bears the scales of the eighth segment in a patch instead of in a single row. Rozeboom and Komp (1950) mentioned a third form, occurring in Colombia, whose male terminalia appear exactly as in *C. (M.) alogistus*. We have found the same form in the highlands of Chiriqui, but have postponed its description while awaiting additional material to make a detailed comparison with typical *alogistus* specimens.

REFERENCE CITED

Rozeboom, L. E., and **W. H. W. Komp.** 1950. A review of the species of *Culex* of the subgenus *Melanoconion* (Diptera, Culicidae). Ann. Ent. Soc. America 43: 75-114.

COMMERCIAL AND EXPERIMENTAL ORGANIC INSECTICIDES

By EUGENE E. KENAGA

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The Immature Stages of the Arctopsychinae Occurring in Eastern North America (Trichoptera: Hydropsychidae)[1]

OLIVER S. FLINT, JR.[2]

Department of Entomology and Limnology, Cornell University, Ithaca, New York

ABSTRACT

Distributional and biological data are presented for *Arctopsyche ladogensis* (Kol.), *A. irrorata* Banks, *Parapsyche apicalis* (Banks), and *P. cardis* Ross, the four arctopsychine species occurring in eastern North America. The larvae and pupae are characterized at the subfamily and the generic levels; and the larvae of all four and the pupae of the two *Parapsyche* species are keyed, described, and illustrated.

Martynov (1924) established the Arctopsychidae as a family to receive the genera *Arctopsyche* and *Arctopsychodes*. Betten (1934) described the third included genus, *Parapsyche*. *Arctopsyche* is a Holarctic genus, but *Arctopsychodes* is exclusively Palaearctic and *Parapsyche* is Nearctic.

The rank accorded to the arctopsychines varies between family and subfamily according to the worker. Martynov (1924), Milne and Milne (1938), and Ulmer (1957), to mention but a few, considered the taxon to be a distinct family. Ross (1956) accorded only subfamily status to the arctopsychines. I am accepting the same status, for the following reasons. The larvae have the typical hydropsychid habitus—large sclerites covering all the thoracic nota, bushy abdominal gills, and anal prolegs and brushes. The large gula is the only major morphological structure limited to the larvae of the arctopsychines. In all the Arctopsychinae this sclerite is large, and divides the genae ventrally over their entire length. However, in *Arctopsyche* it rapidly narrows posteriorly, and in the Diplectroninae only an anterior and a posterior fragment remain. In the Macronematinae and Hydropsychinae the posterior fragment is lost. Thus, quite a complete sequence is present in the Hydropsychidae showing the reduction of the gular sclerite. The pupae also show definite hydropsychid characters—hairy labrum, toothed mandibles, lateral abdominal processes, and apical abdominal processes.

The mature larvae and pupae of the subfamily may be characterized as follows.

LARVA.—Campodeiform. Head roughly quadrate in dorsal aspect; frontoclypeus expanded laterally near the middle (figs. 1, 12, 14, 23); gular sclerite completely separating genae. Labrum about 2.5 times as wide as long; many setae dorsally, and large anterolateral brushes (fig. 10). Mandibles with teeth limited to apical half of inner margin; outer surface with many setae, penicillus mesally on left mandible (fig. 16). Maxillolabium with many setae on sclerites; submentum truncate anteriorly (fig. 2). Pro-, meso-, and metanota each with large sclerotic shields; mes- and metanota each divided by a transverse suture near the middle (figs. 3, 13, 15, 24). Stridulator of fore leg not divided (fig. 7). Prosternite transversely rectangular (fig. 6). Mid and hind legs nearly identical in shape and setal pattern (fig. 5); fore leg shorter and thicker (fig. 4). Neither lateral line nor spacing humps. Gills with filaments clustered at apex of a common stalk; subventral row of gills consisting of paired stalks on abdominal segments 2 to 9 (figs. 27-30). Eighth abdominal sternum with a pair of large submedian plates. Ninth segment with a pair of submedian plates ventrally, another pair of small sublateral plates, and a large pair dorsally. Anal gills five. Anal prolegs extending freely from tenth segment; brush of setae present above anal claw.

PUPA.—Exarate. Labrum semicircular, with many setae. Tibial spurs 2, 4, 4. Hair fringe present on meso- and methathoracic legs. Hook plates anteriorly on segments 3 to 7; posteriorly on 3 and 5 (figs. 21, 26). Gills with filaments clustered at apex of a common stalk. Lateral projections on abdominal segments 3 to 7. Apical processes well developed (figs. 19, 25).

The larvae may be easily separated from the other hydropsychid larvae by two conspicuous characters. In the typical hydropsychids the gula is short and triangular, resulting in the fusion of the genae ventrally over most of their length. The arctopsychines have a large gula that completely separates the genae. The form of the gills is also characteristic; i.e., in the majority of the family the filaments arise from the sides and apex of a common stalk, whereas in the arctopsychines all the filaments are clustered at the apex of the common stalk. The pupae are also immediately separable from the other hydropsychid genera by the presence of hook plates posteriorly on segments 3 and 5. The remainder of the family has plates posteriorly on segments 3 or 4, or both, never on 5.

KEY TO THE LARVAE OF GENERA AND SPECIES FOUND IN EASTERN NORTH AMERICA.

1. Gular sclerite with sides nearly parallel (fig. 18); abdomen with short, wide, scale-like setae on dorsum and in tufts along posterior margin of segments (figs. 17, 22) ..... **Parapsyche** 2
   Gular sclerite narrowed posteriorly to less than ½ its anterior width (fig. 8); abdomen with long, slightly flattened setae, never in tufts (figs. 9, 11) ..... **Arctopsyche** 3

[1] Accepted for publication April 26, 1960.

[2] I wish to express my appreciation to Dr. H. H. Ross and Dr. H. Dietrich, who made material available from their collections, and to the Grace H. Griswold fund of Cornell University which provided funds to meet part of the cost of publishing the plates.

2. Head with a distinctive brown and yellow color pattern (fig. 23); scale tufts on anterior abdominal segments with 2 to 4 scales each (fig. 22) ..... **P. cardis**
Head nearly uniform brown (fig. 14); scale tufts with 6 to 8 scales each (fig. 17) ..... **P. apicalis**
3. Head mostly brown dorsally with a pale, Y-shaped mark roughly following the frontal sutures (fig. 1) ..... **A. ladogensis**
Head mostly dark brown dorsally with eight distinct pale spots (fig. 12) ..... **A. irrorata**

KEY TO THE PUPAE OF GENERA AND KNOWN SPECIES FOUND IN EASTERN NORTH AMERICA.

1. Apical processes elevated to about 90° above body axis, with short, scabrous setae posteriorly (figs. 19, 25); mandibles tubular, with inflated bases and teeth near apex (fig. 20) **Parapsyche** 2
Apical processes only slightly elevated above body axis, bearing many long, stout setae; mandibles gradually widened from apex to base, with teeth over half length of inner margin **Arctopsyche**
2. Apical processes with a thumb-like apicomesal projection (fig. 25); hook plate 5P with two irregular rows of teeth (fig. 26) ..... **P. cardis**
Apical processes with tip bifid (fig. 19); plate 5P with a single irregular row of hooks (fig. 21) ..... **P. apicalis**

Genus **Arctopsyche** McLachlan

Arctopsyche McLachlan, 1868, Trans. Ent. Soc. Lond. 1868: 300.
Genotype, monobasic: *Aphelocheira ladogensis* Kolenati 1859.

McLachlan established *Arctopsyche* in 1868 for *Aphelocheira ladogensis* Kolenati. Since then the genus has grown considerably, and now contains approximately 13 species. Three species are limited to the western montane regions of North America, and one to the Southern Appalachian Region. In addition, the genotype, which is Holarctic in distribution, is found in the Northeast.

The immature stages of several species have previously been described. Silfvenius (1905) gives an excellent description of a Finnish species of the genus that he tentatively placed in *Hydropsyche*. Ulmer (1957, p. 415) stated that Lepneva had found this to be the larva of *A. ladogensis*. Tsuda (1937) described the larvae of *A. maculata* Ulm., and species A and B from Japan. Akagi (1956) redescribed these three forms, as well as forms C and D. However, several of these larvae appear to belong to *Parapsyche* and not *Arctopsyche*. All stages of a Rocky Mountain species, *A. grandis* Bks., were described by Milne and Milne (1938). Considering these descriptions and other material, the genus has the following distinctive characters in the immature stages.

LARVA.—Large, 20 mm. or more in length. Gular sclerite narrowing posteriorly to less than half its width anteriorly (fig. 8). Abdomen without broad, scalelike setae (figs. 9, 11). The two lateral rows of gills with five or more apical filaments (figs. 27, 28).

PUPA.—Mandibles widening rather evenly from apex to base, bearing teeth for nearly half length of inner margin. Apical processes barely raised above axis of body; bearing many long, stout setae.

Arctopsyche ladogensis (Kolenati)

1859 *Aphelocheira ladogensis* Kolenati, Genera et Species Trichopterorum, Pars Altera: 201, pl. 1, fig. 4.
1938 *Arctopsyche ladogensis* (Kol.), Milne and Milne, Bull. Brooklyn Ent. Soc. 33: 103-4, pl. IV.

In North America this species is recorded from the North West Territories, Manitoba, Maine, and New Hampshire. The larvae ascribed to this species have not been reared. However, the association seems quite certain, as this is the only species of *Arctopsyche* known to occur in the Northeast. No conspicuous differences are noticeable between this material and the description of the larvae from Finland (Silfvenius 1905).

LARVA.—Length 20-25 mm, width 2.5-3 mm. Head brown; paler posteriorly, around eyes, and ventrally; with a Y-shaped pale mark roughly following frontal sutures (fig. 1). Muscle scars dark, but indistinct, on posterior. Thoracic nota brown with pale central stripe and dark muscle scars laterally (fig 3). Legs brown. Mesosternum with one pair of gills, metasternum with two pairs. Abdominal gills as in fig. 27. Anal sclerites brown; anal brush of about 40 setae.

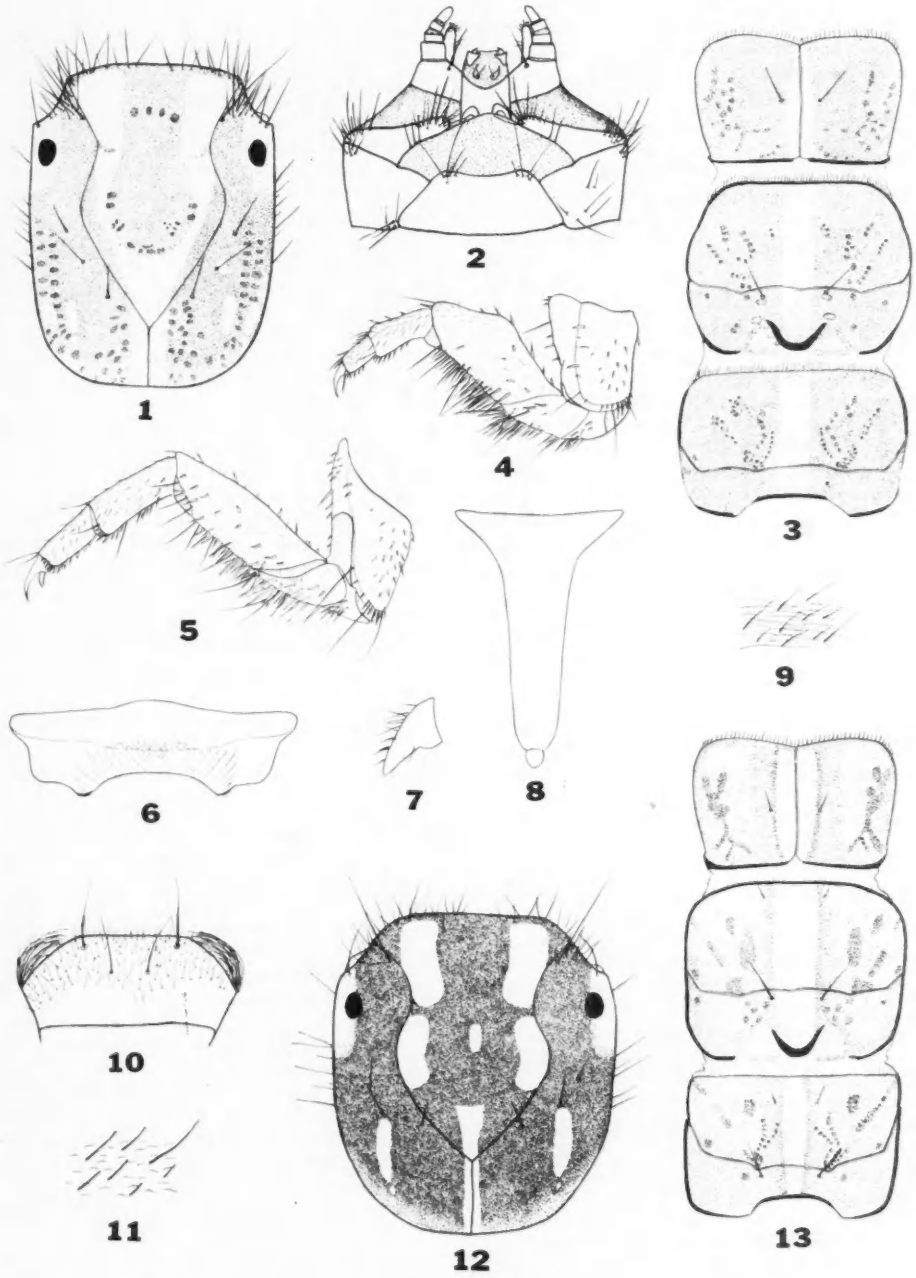
PUPA.—Unknown.

MATERIAL EXAMINED.—*Maine*. Roaring Brook, Mount Katahdin: 8 larvae, VIII-23-57 (Flint). *New Hampshire*. Zealand Stream, near Zealand Campground: 2 larvae, V-18-57 (Flint and Smith). Ammonoosuc River, Zealand Campground: 3 larvae, VIII-3-58 (Flint and Valentine). Fabayan: 1 larva, VIII-3-58 (Flint and Valentine); 5 larvae, VIII-25-57 (Flint, Carson, and Hoeflich). Moose Brook, Gorham: 2 larvae, VIII-8-55 (Flint).

Variation.—The ground color of the head varies considerably in intensity, from near black to brown. The Y-shaped mark may be interrupted near the posterior of the fronto-clypeus where the central bar widens. It is also sometimes broken further anteriorly at the crescent-shaped marks. However, it always appears to the naked eye to have the characteristic Y-shape.

A single small larva, about 7 mm. long, is much paler. Only the Y-mark and the central stripe of the thorax are narrowly outlined in brown.

Biology.—Most of the collections were made in fairly large streams of from 3 to 10 yards wide and 1 to 3 feet deep. All the streams were clear and cold with a bottom of gravel and boulders. The larvae are generally widely scattered over the stream. However, the collection from Mount Katahdin, Maine, comes from several long streaming branches of water moss, *Fontinalis*. The larvae build a large, strong retreat and net typical of the family. The retreats are generally attached to some small depression in the side of the boulder where the net can extend into the full force of the current. Those living in the water moss were in the strongest current.



[PLATE 1]

FIGS. 1-10.—Larval structures of *Arctopsyche ladogensis* (Kol.). FIG. 1.—Head, dorsal. FIG. 2—Maxillolabium, ventral. FIG. 3.—Thoracic nota, dorsal. FIG. 4.—Fore leg, posterior. FIG. 5.—Middle leg, posterior. FIG. 6.—Prosternite, ventral. FIG. 7.—Fore trochantin, lateral. FIG. 8.—Gula, ventral. FIG 9.—Portion of abdomen, showing typical arrangement of hairs and setae. FIG. 10.—Labrum, dorsal.

FIGS. 11-13.—Larval structures of *Arctopsyche irrorata* Banks. FIG. 11.—Portion of abdomen, showing typical arrangement of hairs and setae. FIG. 12.—Head, dorsal. FIG. 13.—Thoracic nota, dorsal.

The smallest larva, 7 mm. long, was taken on August 3. The full-grown larvae were taken in late August and May, indicating that the species probably overwinters as mature larvae. Pupation occurs in late May or June, and the few published adult records are all in June.

Arctopsyche irrorata Banks

1905 *Arctopsyche irrorata* Banks, Bull. Amer. Mus. Nat. Hist. 21:217.
1938 *Arctopsyche irrorata* Banks, Milne and Milne, Bull. Brooklyn Ent. Soc. 33: 103, pl. IV.

This species has only been found in North and South Carolina but may be expected in appropriate stations in Tennessee and Georgia. The association of larva and adult is based on the distribution of the species.

LARVA.—Length 23-27 mm., width 3-4 mm. Head black; paler laterally, around eyes, and midventrally; a pattern of distinct pale spots dorsally (fig. 12). Thoracic nota with pale central line, and laterally with an irregular dark pattern (fig. 13). Legs brown. Mesosternum with one pair of gills, metasternum with two pairs. Gills on abdomen as in fig. 28. Anal sclerites brown; anal brush of over 50 setae.

PUPA.—*Unknown.*

MATERIAL EXAMINED.—*North Carolina.* Oconaluftee River, Smokemont, Great Smoky Mountains National Park: 3 larvae, VII-2-58 (Flint and Hanson). Mingus Creek, Great Smoky Mountains National Park: 13 larvae, IX-13-58 (Flint and Wiggins). Clear Creek, near Highlands: 2 larvae, VII-4-58 (Flint); 30 larvae, IX-10-58 (Flint and Wiggins). Looking Glass Creek, Pisgah National Forest: 4 larvae, VII-5-58 (Flint). *South Carolina.* East Fork Chattooga River, Walhalla Federal Fish Hatchery: 50 larvae, IX-11-58 (Flint and Wiggins).

Variation.—The mature larvae seem to be constant in color pattern.

The smallest larvae, around 6 mm. long, are very pale. A Y-shaped mark is present on the head, much as in the small larva of *ladogensis*. However, there is an additional pale spot posterior to the eye, and a dark sublateral bar ventrally in *irrorata*.

Biology.—The species seems to frequent the same type of streams as does its northern counterpart. The larvae often are very abundant in shallow water where a stream drops rapidly over bedrock. In these conditions any crevice in the rock is outlined with a row of retreats. Other larvae are found attached to irregularities in boulders in the swiftest parts of the current.

Small larvae, 6 to 8 mm. in length, were taken in early July. By September the larvae were fully grown. Published adult records fall in late May and June, showing that the species probably also overwinters as mature larvae.

Genus **Parapsyche** Betten

Parapsyche Betten, 1934, New York State Mus. Bull. 292: 181.
Genotype, monobasic: *Arctopsyche apicalis* Banks 1908.

Established in 1934 for *Arctopsyche apicalis* Banks, the genus now contains six species and is definitely known only from the Nearctic Region. However, some of the larvae described from Japan by Akagi (1956) apparently belong to this genus. The species are easily divisible into two groups based on the male genitalia, with one species from each group known from eastern North America. The record of a third species, *P. elsis* Milne and Milne, from North Carolina by Milne and Milne (1938) is undoubtedly the result of a misdetermination of the closely related *P. cardis* Ross.

Ross (1944) has figured some of the characters of the immature stages of *P. cardis*. A study of the two eastern species shows the following characters distinctive for the immature stages of this genus.

LARVA.—Smaller, rarely reaching 20 mm. in length. Gular sclerite nearly parallel-sided (fig. 18). Abdomen with many scattered scalelike setae; tufts of these setae on posterior dorsal margins of the segments (figs. 17, 22).

PUPA.—Mandibles rather tubular, widening suddenly at bases; teeth borne apically (fig. 20). Apical processes recurving to an angle of 90° or more to axis of body; with short, scabrous setae posteriorly and short setae basally (figs. 19, 25).

Parapsyche apicalis (Banks)

1908 *Arctopsyche apicalis* Banks, Trans. Amer. Ent. Soc. 34: 266.
1934 *Parapsyche apicalis* (Banks), Betten, New York State Mus. Bull. 292: 181-82, pl. 16, figs. 14-18, pl. 17, figs. 1-2.
1938 *Parapsyche apicalis* (Banks), Milne and Milne. Bull. Brooklyn Ent. Soc. 33; 105-06, pl. V.

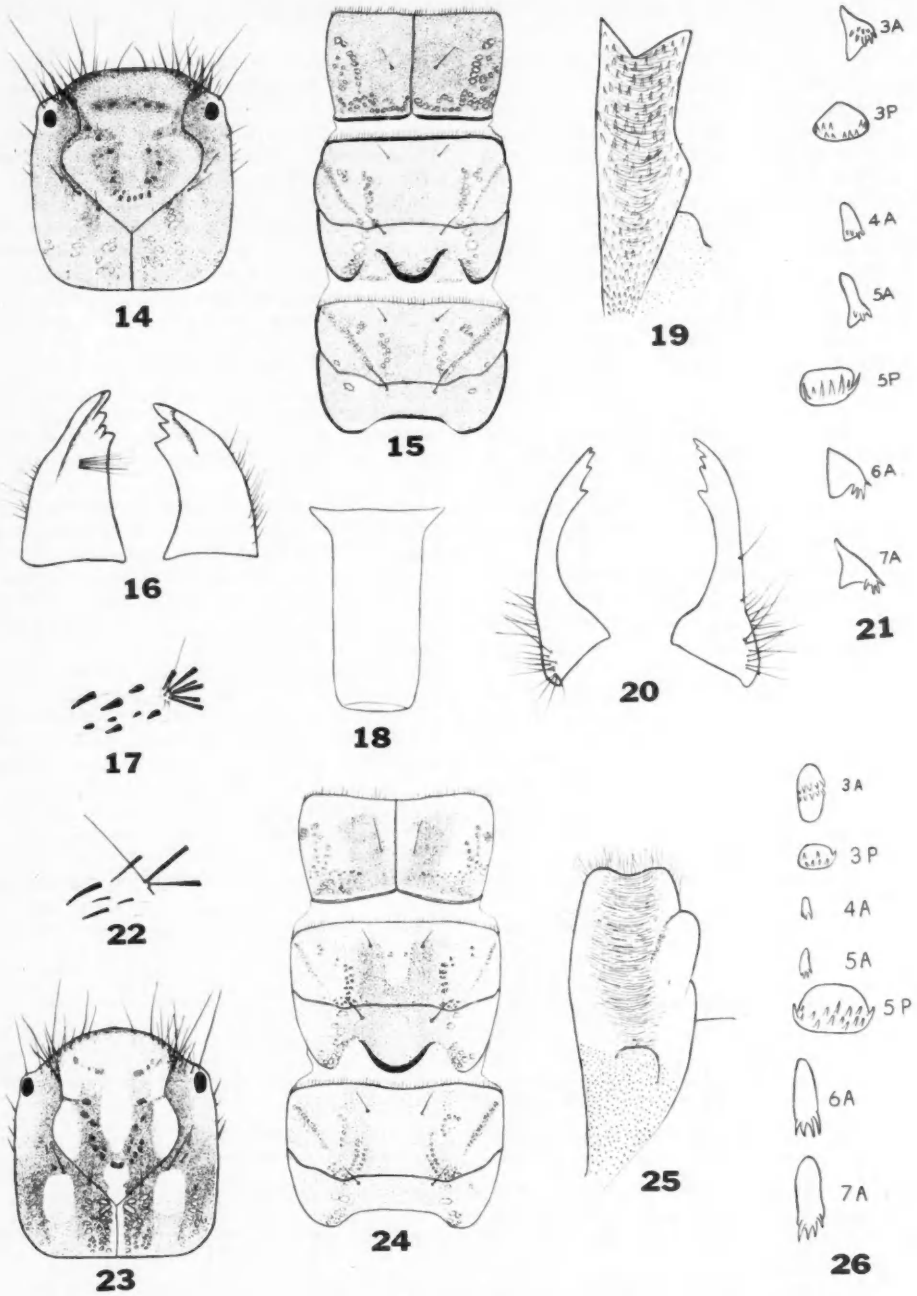
The species has a wide range in the East, being known from North Carolina, Tennessee, Virginia, New York, Massachusetts, New Hampshire, Nova Scotia, Ontario, and Michigan. The Colorado record of Milne and Milne (1938) probably refers to *P. almota* Ross, a western species which is difficult to separate from *apicalis*.

The association of adult and larva is based on a mature male pupa taken in Sunderland, Massachusetts.

LARVA.—Length 18-20 mm., width 2.5-3 mm. Head brown; slightly paler around eyes and ventrally; muscle scars inconspicuously darker (fig. 14). Thoracic nota and legs brown (fig. 15). Mesosternum with one pair of gills, metasternum with two pairs. Abdominal segments 1 to 7 dorsally with two pairs of scale tufts, segment 8 with one pair; most tufts with 6 to 8 scales and 1 to 2 normal setae (fig. 17). Gills as in fig. 29. Anal sclerites yellow brown; anal brush of about 10 setae.

PUPA.—Length 9-10 mm., width 2-3 mm. Hook plate 5P with a single irregular row of hooks (fig. 21). Apical processes with tips bifid; posterior face with many short, pointed setae (fig. 19).

MATERIAL EXAMINED.—*Maine.* Roaring Brook, Mount Katahdin: 2 larvae, VIII-23-57 (Flint). *Massachusetts.* Fish Hatchery, Sunderland: 5 lar-



[PLATE 2]

FIGS. 14-18.—Larval structures of *Parapsyche apicalis* (Banks). FIG. 14.—Head, dorsal. FIG. 15.—Thoracic nota, dorsal. FIG. 16.—Mandibles, posterior. FIG. 17.—Portion of abdomen, showing typical arrangement of scales, and one scale tuft. FIG. 18.—Gula, ventral. FIGS. 19-21.—Pupal structures of *P. apicalis*. FIG. 19.—Left apical process, posterior. FIG. 20.—Mandibles, posterior. FIG. 21.—Hook plates, dorsal, of abdominal segments 3 to 7; A, anterior on segment, P, posterior.

FIGS. 22-24.—Larval structures of *Parapsyche cardis* Ross. FIG. 22.—Portion of abdomen showing typical arrangement of scales, and one scale tuft. FIG. 23.—Head, dorsal. FIG. 24.—Thoracic nota, dorsal. FIGS. 25-26.—Pupal structures of *P. cardis*. FIG. 25.—Left apical process, posterior. FIG. 26.—Hook plates, dorsal; abbreviations as in fig. 21.

vae, II-9-55 (Flint); 2 larvae, VI-17-58 (Flint); 3 larvae, VII-6-57 (Flint); 3 larvae, VII 9-54 (Flint); 2 larvae, 3 pupae; VIII-6-54 (Flint); 4 pupae, VIII-28-56 (Flint); 3 larvae, XI-2-53 (Flint); 5 larvae, XI-9-53 (Flint). Montague: 9 larvae, II-7-59 (Flint); 7 larvae, IV-2-58 (Flint). *New Hampshire.* Cutler River, Tuckerman's Ravine, Mount Washington: 7 larvae, VIII-4-58 (Flint and Valentine); 10 larvae, VIII-24-57 (Flint, Carson, and Hoeflich). Cutler River, Pinkham Notch: 1 larva, VIII-4-58 (Flint and Valentine). *North Carolina.* Indian Gap, Great Smoky Mountains National Park: 4 larvae, VII-1-58 (Flint and Hanson). *Ontario.* Kendal: 5 larvae, X-12-57 (Flint). *Tennessee.* Indian Gap, Great Smoky Mountains National Park: 1 larva, 1 pupa, IX-13-58 (Flint and Wiggins). *Virginia.* Hogcamp Brook, Shenandoah National Park: 1 larva, III-28-57 (Flint); 5 larvae, IX-18-58 (Flint and Wiggins). White Oak Canyon, Shenandoah National Park: 3 larvae, III-28-57 (Flint). Hawksbill Spring, Shenandoah National Park: 2 larvae, III-28-57 (Flint).

Variation.—There is almost no variation in the immatures of this species. A few specimens show a slightly more or less prominent color pattern than the one figured. All larval instars have the same pattern.

Biology.—The species is commonly found in cold (generally below 50° F.), spring-fed brooklets only a few feet wide; occasionally they may be found in rushing mountain brooks several yards wide. The larvae construct a typical retreat and net attached to sticks and stones in the full current. During the fall and winter they are sometimes found on the fallen leaves that may clog the stream.

The pupal cases are almost exclusively organic matter. Only rarely does *Parapsyche apicalis* use sand grains, as *Hydropsyche* habitually does. The pupae are completely enclosed in the case, the substrate not closing one side as in *Hydropsyche.*

The following seasonal history is found in Sunderland, Massachusetts, and is probably typical. The winter is passed as larvae in many instars. Pupae are found throughout most of the summer, as are the adults. In 1954, as an example, adults were collected from May 11 to October 1. It is possible that there are several generations a year, but without definite broods.

Parapsyche cardis Ross

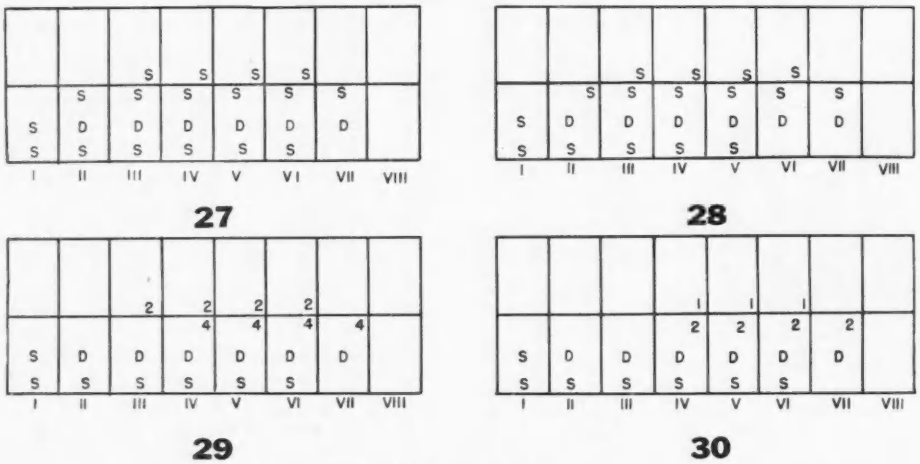
1938 *Parapsyche cardis* Ross, Proc. Ent. Soc. Washington 40: 119, fig. 4.

Known from Georgia, North Carolina, and Tennessee, this species may be expected also in South Carolina.

The correlation of adults and larvae is based on determined larvae supplied by H. H. Ross, to whom I am very grateful, and associated pupal exuviae in the Betten Collection at Cornell. Ross (1944) illustrated certain characters of the immature stages of this species.

LARVA.—Length 18-20 mm., width 2.5-3 mm. Head pale yellow with dark brown markings (fig. 23); ventrally with dark sublateral bars. Thoracic nota pale with dark submedial bars and muscle scars (fig. 24). Legs yellow. Mesosternum with a single pair of gill tufts, metasternum with two pairs. Abdominal scale tufts on segments 2 to 5 with only 2 to 4 scales per tuft (fig. 22). Gills as in fig. 30. Anal sclerites yellow; anal brush of about 10 setae.

PUPA.—Length 12-14 mm., width 2.5-3 mm. Hook plate 5 P with two irregular rows of hooks (fig. 26).



[TEXT FIGURE]

FIGS. 27-30.—Gill diagrams (schematic representation of left side of larva). I to VIII, abdominal segments 1-8; 1, 2, or 4, number of apical filaments on a stalk; S, five or more filaments on a stalk; D, two S-type gills arising contiguously. FIG. 27.—*Arctopsyche ladogensis.* FIG. 28.—*A. irrorata.* FIG. 29.—*Parapsyche apicalis.* FIG. 30.—*P. cardis.*

Apical processes with thumblike mesal projection; with small pointed setae and short hairs basally (fig. 25).

MATERIAL EXAMINED.—*Georgia.* Black Rock Mountain State Park, Rabun County: 2 larvae, VII-3-58 (Flint and Hanson); 50 larvae, IX-10-58 (Flint and Wiggins). *North Carolina.* Black Mountain: 2 exuviae, V-21-52 (Betten). Near Flat Top Mountain, Swannanoa: 1 exuvium, VI-7-52 (Betten). Clear Creek, near Highlands: 4 larvae, VII-4-58 (Flint). Biological Station, Highlands: 21 larvae, IX-10-58 (Flint and Wiggins). Camp Alice, Mount Michell: 30 larvae, IX-10-58 (Flint and Wiggins). Route 221, near Linville: 10 larvae, IX-17-58 (Flint and Wiggins).

Variation.—Each larval instar has a rather consistent and distinct coloration. The smallest larva, about 6 mm. long, is almost entirely pale yellow. However, the head bears two pairs of darker marks, one centrally on the frontoclypeus, the other immediately behind this on the genae. As the larvae mature the marks become larger and darker and coalesce, and the dark lateral areas become prominent. The dark pattern of the thorax does not become prominent until the last or the next-to-last instar.

Biology.—This species inhabits streams of the same type as *P. apicalis*, and shows the same habits. Even though both species inhabit North Carolina, there appears to be a habitat separation of the two. *P. apicalis* is found only in the Smoky Mountains at elevations of about 5,000 feet; yet on Mt. Mitchell only *cardis* was taken at 5,700 feet. However, Mt. Mitchell is cut over and the stream is mostly in the open, with a resulting temperature in the mid-fifties in September.

The larvae taken in early July were all small, from 6 to 7 mm. in length, while those taken in September were much larger and many were full-grown. Fully grown larvae have also been collected in May, which indicates that the species overwinters as mature larvae. In Highlands, N. C., adults were collected by J. G. Franclemont from June 26 to September 5, 1958.

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A Study of Bisexual and Parthenogenetic Strains of *Pycnoscelus surinamensis* (Blattaria : Epilamprinae)[1]

LOUIS M. ROTH[2] AND EDWIN R. WILLIS[2]

Pioneering Research Division, U. S. Army
Quartermaster Research and Engineering Center,
Natick, Massachusetts

ABSTRACT

The bisexual strain of *Pycnoscelus surinamensis* from Hawaii differs biologically and morphologically from the parthenogenetic strain of *P. surinamensis* from Florida. The former does not successfully reproduce parthenogenetically, primarily because virgin females fail to retract their oöthecae normally. Only seven out of several hundred virgin females of this strain succeeded in transferring their oöthecae into their brood sacs, and none of these eggs hatched. About 70% of the mated females of this strain retracted their oöthecae normally, and subsequently gave birth to male and female offspring. Virgin females of *P. surinamensis* from Florida reproduce parthenogenetically. Females of this parthenogenetic strain that were mated to males of the bisexual form showed reduced fertility, and, although many of them were found to have sperm in their spermathecae, all of their offspring were females—which is typical of the parthenogenetic form—and no male offspring were produced. There are statistically significant differences between females of the two strains in several linear measurements and in ratios based on these measurements.

Pycnoscelus surinamensis (Linnaeus), the intermediate host of the chicken eye worm, is parthenogenetic wherever it has become established in the Western Hemisphere. Males of this species apparently have never been found in nature in the New World although males, referred to as *P. surinamensis*, are encountered in oriental collections (Rehn 1945.).

In 1950 we started a laboratory colony of *Pycnoscelus surinamensis* from two females collected in Clearwater, Florida. Over the intervening years a large culture of these parthenogenetic females was maintained. In 1954 Fred A. Bianchi shipped us some living *Diploptera punctata* (Eschscholtz) from Hawaii; inadvertantly included with them were cockroach nymphs of another species which matured into males and females of what appears to be the bisexual form of *P. surinamensis*.

In connection with the present study Dr. Ashley Gurney (1958) examined specimens of the Hawaiian bisexual form of *Pycnoscelus* and stated: "To the best of my knowledge, they are *P. surinamensis* (L.)." Dr. K. Princis (1959) wrote us, "as far as I can judge both the samples (from Florida and Hawaii) you sent belong to *Pycnoscelus surinamensis*." Dr. J. A. G. Rehn (1958) accepted males of the Hawaiian bisexual species as *P. surinamensis*. All three specialists based their opinions on the usual morphological characters and were, at that time, unaware of our biological studies. However, Dr. Princis, after learning of our observations, reexamined the material we had sent him and concluded that the Hawaiian bisexual form of *Pycnoscelus* is a new species. Although it is conceivable that two species are involved, we have adopted a more conservative view and retained both strains in one species. This aspect of the problem is treated more fully in the discussion.

According to Zimmerman (1948), *Pycnoscelus surinamensis* was the first cockroach recorded from Hawaii by name (*Blatta punctata* of Eschscholtz, 1822). However, Princis (1950) pointed out that Kirby was incorrect in synonymizing *B. punctata* under *P. surinamensis*. From Eschscholtz's description Princis concluded that *B. punctata* was actually *Diploptera punctata*. The only species of *Pycnoscelus* listed for Hawaii by Zimmerman (1948) is *surinamensis*; the adult individual shown in figure 49 of his publication is a male, undoubtedly the male of the bisexual form that we are dealing with in this study. Zimmerman (1948) stated that *P. surinamensis* was ". . . reported to be parthenogenetic in some localities" (presumably referring to some areas in the Hawaiian Islands).

Initially, we tried to keep the Hawaiian and Floridian cultures separate. But when males from the bisexual colony became quite numerous, a few stray males were found among the parthenogenetic females; hence we assumed (incorrectly, as it turned out) that the two forms had become genetically mixed, and we made no further attempts to keep them isolated. However, recently, while studying factors affecting the development of oöcytes in several species of cockroaches, we found that the parthenogenetic and bisexual forms of *Pycnoscelus* differed physiologically and that the bisexual form could not reproduce parthenogenetically in the laboratory. This paper reports the results of our study of the bisexual (Hawaiian) and parthenogenetic (Floridian) forms of *Pycnoscelus surinamensis*.

OBSERVATIONS

Biological.—Some biological data for both strains are given in table 1. In the Hawaiian bisexual strain the rate of growth of the oöcytes in virgin females was retarded after the 6th day, whereas mating caused the oöcytes to grow more rapidly (fig. 1A). In virgin parthenogenetic females there was no noticeable retardation in the rate of growth of the oöcytes (fig. 1C). The mean size of the mature oöcyte of the parthenogenetic strain was significantly

[1] Accepted for publication May 10, 1960.
[2] Present address: United Fruit Company, Central Research Laboratories, Upland Road, Norwood, Massachusetts.

larger than that of the bisexual form ($P < 0.001$). Mated females of the bisexual strain oviposited significantly sooner than virgins ($P < 0.001$); the peak of the oviposition curve of mated females occurred at 11 days and that of virgins at 13 days (fig. 1B).[3] The mating of parthenogenetic strain females with bisexual males did not result in a significant change in the preoviposition period from that of virgin parthenogenetic females (table 1); the peaks of the oviposition curves of both occurred at 12 days (fig. 1D; cf. fig. 1B).

and 7 had sperm. Two females retracted the oötheca only half-way into the brood sac, leaving about half of the oötheca protruding beyond the abdomen; both of these females had sperm in their spermathecae.

Of 138 virgin bisexual females only two individuals retracted their oöthecae normally (table 2). All others dropped their oöthecae soon after formation; these unretracted oöthecae were incompletely formed (fig. 2B; cf. fig. 2A). Frequently, the last-laid eggs were disarranged and were not covered with oöthecal secretion (fig. 2C, top). Thirty-six virgin bisexual females were examined after dropping their oöthecae; 19 had laid all their mature oöcytes in their ovaries. Of hundreds of additional virgin females that we observed, only five more individuals retracted their

Table 1.—Biological data[a] for the bisexual and parthenogenetic strains of *Pycnoscelus surinamensis*. N = number of individuals; S.E. = standard error.

Biological observation	Bisexual				Parthenogenetic			
	Min.	Max.	Mean ± S.E.	N	Min.	Max.	Mean ± S.E.	N
Size (mm.) of mature oöcyte at oviposition	2.69	3.36	2.99 ± 0.04	15	2.97	3.36	3.21 ± 0.04	10
Preoviposition period (days):								
Virgin	8	25	13.6 ± 0.2	138	10	20	12.8 ± 0.1	244
Mated	9	22	11.9 ± 0.1	59	10	14	12.5 ± 0.2	26[b]
Gestation period (days):								
Virgin	—	—	—	—	53	58	55.4 ± 0.3	20
Mated	50	56	52.8 ± 0.2	37	50	58	54.8 ± 0.4	16[b]
Number eggs hatched per oötheca:								
Virgin	0	0	0	7[c]	0	30	20.7 ± 1.3	29[d]
Mated	5	37	24.5 ± 1.7	28	0	16	6.2 ± 1.3	15[b, e]
					2	11	5.4 ± 1.0	11[b, f]
					0	8	2.2 ± 1.2	6[b, g]
No. of days to oviposition after parturition:								
Virgin	—	—	—	—	14	17	15.5 ± 0.3	11
Mated	10	16	14.2 ± 0.7	8	—	—	—	—

[a] Throughout this study insects were kept at 24°-25° C. and 50%-70% R.H., and were fed Purina Dog Chow Checkers.
[b] Parthenogenetic females from Florida that were mated to males of the bisexual strain of *Pycnoscelus* from Hawaii.
[c] These seven individuals (which include the two females in table 2) were the only virgin females of this strain that managed to retract their oöthecae into the uterus, where they were retained for 54 to about 70 days. (The eggs in oöthecae that are not retracted but dropped by females do not develop.) There was noticeable egg development in only one oötheca, which contained several enlarged eggs; one of these eggs contained a very small embryo which did not mature, indicating that some parthenogenesis may occur very rarely in the bisexual strain.
[d] Mean value from Willis et al. 1958; the minimum and maximum values were obtained from their unpublished raw data.
[e] Based on first litters.
[f] Based on second litters.
[g] Based on third litters.

The gestation period of mated bisexual females was slightly less than that of either virgin or mated parthenogenetic females (table 1).

The most striking physiological differences between the parthenogenetic and the bisexual strains were in their mating and ovipositional behaviors and in the sexes of the offspring resulting from mating. The effect of mating on the ability of females to retract the oötheca into the uterus is shown in table 2. Of 74 bisexual females exposed to males, 59 (80%) mated prior to ovipositing. Of the 59 mated females, 41 oviposited normally. Sixteen mated females dropped their oöthecae without retracting them into the brood sacs; 15 of these were examined for the presence of sperm in the spermathecae; 8 lacked sperm oöthecae into their brood sacs; however, none of the eggs hatched in the seven oöthecae that had been retracted by these females (see table 1, footnote c).

Of 248 virgin parthenogenetic females 98% retracted their oöthecae normally (table 2). (See Roth and Willis 1954, plate 11, figures 74-79, for photographs of this species in the act of ovipositing—i.e., formation and retraction of the oötheca.) Two females partially retracted their oöthecae; one dropped her oötheca; and one failed to oviposit, although her oöcytes had matured. Of 42 parthenogenetic females, each exposed to males of the bisexual strain, in 250 ml. beakers, only 26 (62%) mated. All but one of the mated females oviposited normally (table 2); the exceptional female failed to retract her oötheca

[3] We have also observed this effect of mating on initiation of oviposition in other species of cockroaches, e. g., *Blattella germanica* (L.), *Leucophaea maderae* (Fabr.), and *Nauphoeta cinerea* (Oliv.).

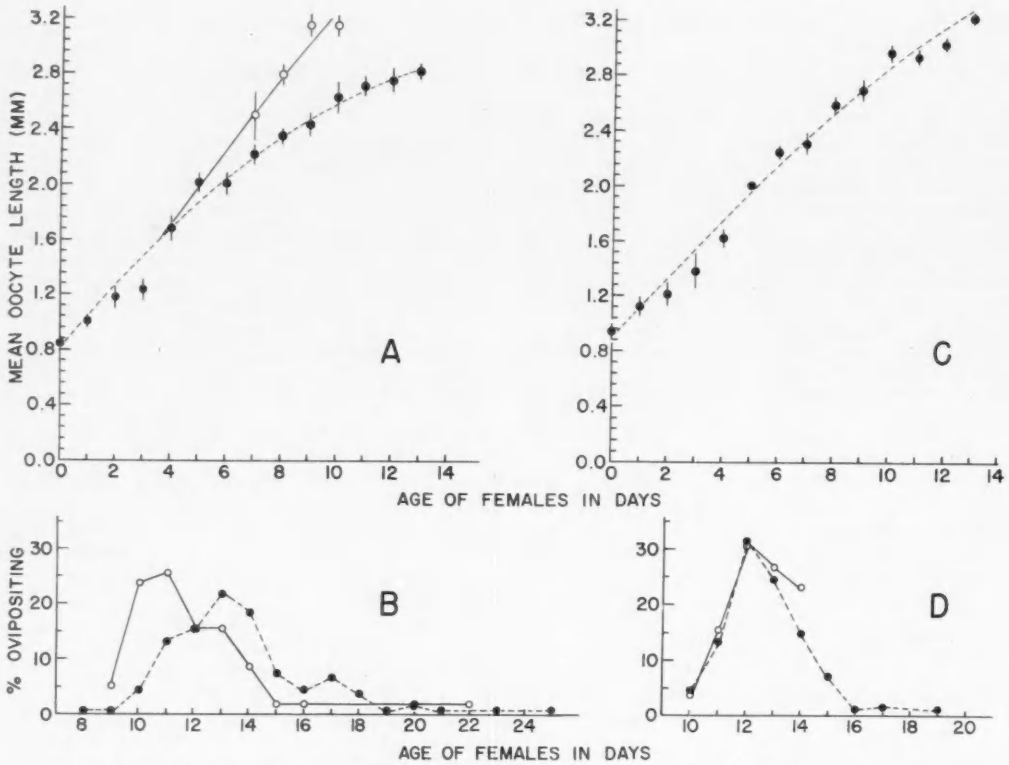

FIG. 1.—Effect of mating on rate of development of oöcytes and age at oviposition in *Pycnoscelus surinamensis;* insects kept at 24° to 25° C. Solid circles, virgin females; open circles, mated females; vertical bars, standard errors. A.—Bisexual strain from Hawaii: 220 virgin females (10 to 20 measurements per mean), 14 females mated when 3 to 5 days old (3 or 4 measurements per mean). B.—Bisexual strain from Hawaii: 138 virgin females, 59 mated females. C.—Parthenogenetic strain from Florida: 134 females (6 to 13 measurements per mean). D.—Parthenogenetic strain from Florida: 244 virgin females, 26 females mated to males of the bisexual strain from Hawaii.

completely. Thus, mating with males of the bisexual strain had no effect on formation of the oötheca (fig. 2C, bottom), or on its retraction into the uterus by females of the parthenogenetic strain.

We noted that when confined in 250 ml. beakers, bisexual males of *Pycnoscelus* made numerous attempts to mate with females of the parthenogenetic strain, but these females tended to resist the males, and only a comparatively few mated. Earlier, we had noticed this reluctance to mate on the part of parthenogenetic females but had managed to obtain photographs of a mating pair (Roth and Willis 1958, plate 22, figures 4-8). The mating behavior (i.e., positions prior to and during copulation) of the

Table 2.—Oviposition by the bisexual and parthenogenetic strains of *Pycnoscelus surinamensis.*

Ovipositional behavior	Number of females observed ovipositing			
	Bisexual		Parthenogenetic	
	Mated	*Virgin*	*Mated*[c]	*Virgin*
Normal[a]	41 (69.5%)	2 (1.4%)	25 (96%)	244 (98%)
Abnormal[b]	18 (30.5%)	136 (98.6%)	1 (4%)	4 (2%)
Totals	59	138	26	248

[a] Females which retracted their oöthecae into the uterus.
[b] Females which dropped their oöthecae at time of oviposition or failed to retract them completely into the uterus.
[c] Parthenogenetic females from Florida that were mated to males of the bisexual strain of *Pycnoscelus* from Hawaii.

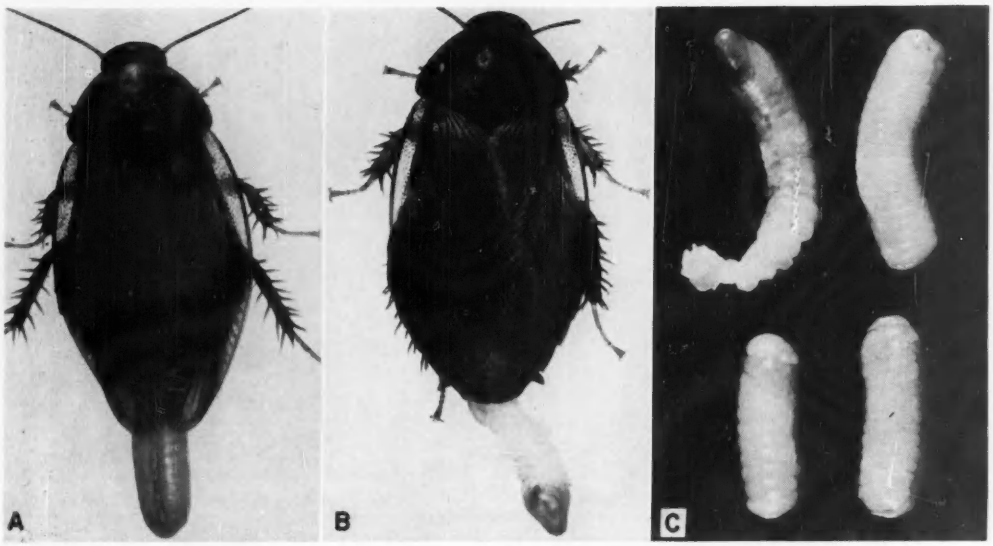

FIG. 2.—A, B, *Pycnoscelus surinamensis*, bisexual strain from Hawaii. A—Mated female forming a normal oötheca which was later retracted into the uterus. B.—Virgin female forming an abnormal oötheca which was later dropped. C.—Oöthecae of *P. surinamensis*. Top: bisexual strain; left, from virgin female; right, from mated female. Bottom: parthenogenetic strain; left, from virgin female; right, from female mated to male of the bisexual strain.

males is the same towards bisexual or parthenogenetic females. As we pointed out earlier (Roth and Willis 1958), the precopulating position in *Pycnoscelus* is unique among cockroaches in that the male is on the back of the female rather than vice versa. To determine whether females of the parthenogenetic strain tend to resist the males of the bisexual form in large cultures, pregnant females (i.e., females bearing oöthecae in their brood sacs) of both the bisexual and parthenogenetic forms were taken from cages in which both strains were numerous, including many males. The spermathecae of 60 females of each strain were examined; sperm was present in all of the pregnant bisexual females but in only 1 of the 60 pregnant parthenogenetic individuals. From another container which had a total population of 184 parthenogenetic females, 12 bisexual females, and 13 bisexual males, all 12 of the bisexual females contained sperm, but only 13 of 20 parthenogenetic females that were examined had sperm in their spermathecae.

It would appear from these observations that, although the bisexual male is capable of mating with the parthenogenetic female, sexual isolation existed even in the relatively confined quarters of the cages (measuring 12″ x 18″ x 7″) whenever large numbers of females of both strains were present. However, if the predominant females in the cage population are parthenogenetic, or if a parthenogenetic female is confined in a small container with a male of the bisexual strain, then the number of parthenogenetic females that mate will increase.

Mating markedly reduced the fertility of parthenogenetic females. An average of 21 eggs per oötheca hatched from unmated females as compared to 6 from mated parthenogenetic females (table 1). It is noteworthy that all the offspring of mated parthenogenetic mothers were females. No males ever resulted from matings between parthenogenetic females and bisexual males. But of 978 offspring from 28 mated bisexual females, 504 were females and 473 were males, a sex ratio of 1 ♀ :0.94 ♂.

About 20% of 142 female offspring mated parthenogenetic females had abnormal abdominal segmentation. These anomalies were restricted to the tergites, which were generally asymmetrical (fig. 3). Rarely, the thorax was affected (fig. 4 G-I). A large number of female offspring of mated bisexual females and of parthenogenetic females that had never been exposed to males were examined for aberrant tergites. The results are shown in table 3. None of the offspring of these bisexual females showed anomalous segmentation, but 12% of the offspring of the unmated parthenogenetic females had anomalies similar to those found in offspring of mated parthenogenetic females. This anomalous segmentation apparently has no casual connection with mating. In January 1960, one of us (L.M.R.) collected a nymph of the parthenogenetic strain of *P. surinamensis* in La Lima, Honduras, which also had anomalous abdominal segmentation; this aberration may be common in the parthenogenetic strain.

All of the biological observations described above were made on parthenogenetic females descended from insects obtained from Florida in 1950 and on bisexual individuals descended from insects obtained

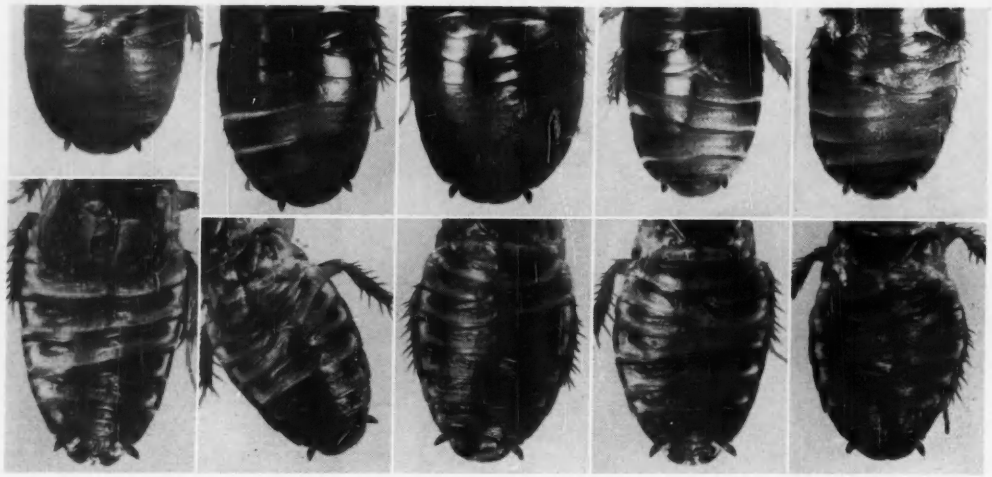

FIG. 3.—*Pycnoscelus surinamensis*, parthenogenetic strain from Florida. Abdomens of females with aberrant tergites. Top row, nymphs; bottom row, adults (wings removed).

from Hawaii in 1954. However, in October 1958, 100 adult females of *Pycnoscelus* were shipped to us from Hawaii by Fred Bianchi; all of the specimens had been collected in ground litter under *Prosopis juliflora* in the Koko Head region of Hawaii. Of these, 98 were typical bisexual individuals, which eventually produced male and female offspring, but two were morphologically similar to the Floridian parthenogenetic strain. One of these latter died shortly after receipt without giving birth. The second parthenogenetic-type female gave birth to one nymph, which died; the other eggs in the oöthecae failed to hatch. This female later bore young two more times yielding five and two living nymphs, respectively. These nymphs matured into females that were morphologically identical to the Floridian parthenogenetic strain, and they reproduced parthenogenetically. The reduced fertility of this one parent parthenogenetic female from Hawaii strongly resembled that of Floridian *P. surinamensis* that had been mated to males of the Hawaiian bisexual form. It is conceivable that this female, having been collected in the same microhabitat with the bisexual strain, may have mated in nature with a bisexual male. Another shipment of living material from Barbers' Point, some 20 miles from Koko Head, Hawaii, contained only bisexual individuals. The insects from Koko Head and Barbers' Point were collected a half-mile or more from human habitations. Several specimens collected in chicken manure at Waianae, Oahu, and one specimen from Waimanalo were all bisexual males.

Table 3.—Incidence of aberrant tergal segmentation in female offspring (nymphs and adults) of *Pycnoscelus surinamensis*.

Types of female parents	Female offspring examined	No. with anomalous tergites	No. with normal tergites
Parthenogenetic[a], never exposed to males of the bisexual strain	331	40 (12%)	291 (88%)
Parthenogenetic (Floridian strain), mated to males of bisexual Hawaiian strain	142	28 (20%)	114 (80%)
Mated females of bisexual strain	504	0 (0%)	504 (100%)

[a] These specimens were received from Dr. L. A. Hetrick from Florida in 1958 and were kept isolated from males of the bisexual strain from Hawaii.

Morphological.—The Hawaiian bisexual strain of *Pycnoscelus surinamensis* differs morphologically from the Floridian parthenogenetic strain. Various differences are summarized in tables 4 and 5. Half of the measured specimens of parthenogenetic females were descendants of our original colony, and half were from a new colony sent to us in 1958 from Gainesville, Florida, by Dr. L. A. Hetrick. Half of the specimens of the Hawaiian bisexual strain were from the colony introduced in 1954, and half were from a new colony collected in 1958 by Fred Bianchi. Thus, the specimens of each form, although collected at different times, came from two populations that are breeding in relatively restricted geographical areas, either in Florida or in Hawaii. Undoubtedly each population is homogeneous within its respective area and exhibits less variation in morphological characters than may exist between it and another population of the same species breeding in a noncontiguous area. These morphological differences between populations may possibly characterize sibling species or geographical varieties, as suggested by museum specimens that exhibit variable characters (see below).

The most conspicuous morphological difference between the two strains under discussion is the reduction in the size of the wings and tegmina of the bisexual strain in relation to its overall length (cf. figs. 5A-C and 5D-F). This is particularly noticeable in living animals (cf. figs. 5C and 5F). In dried

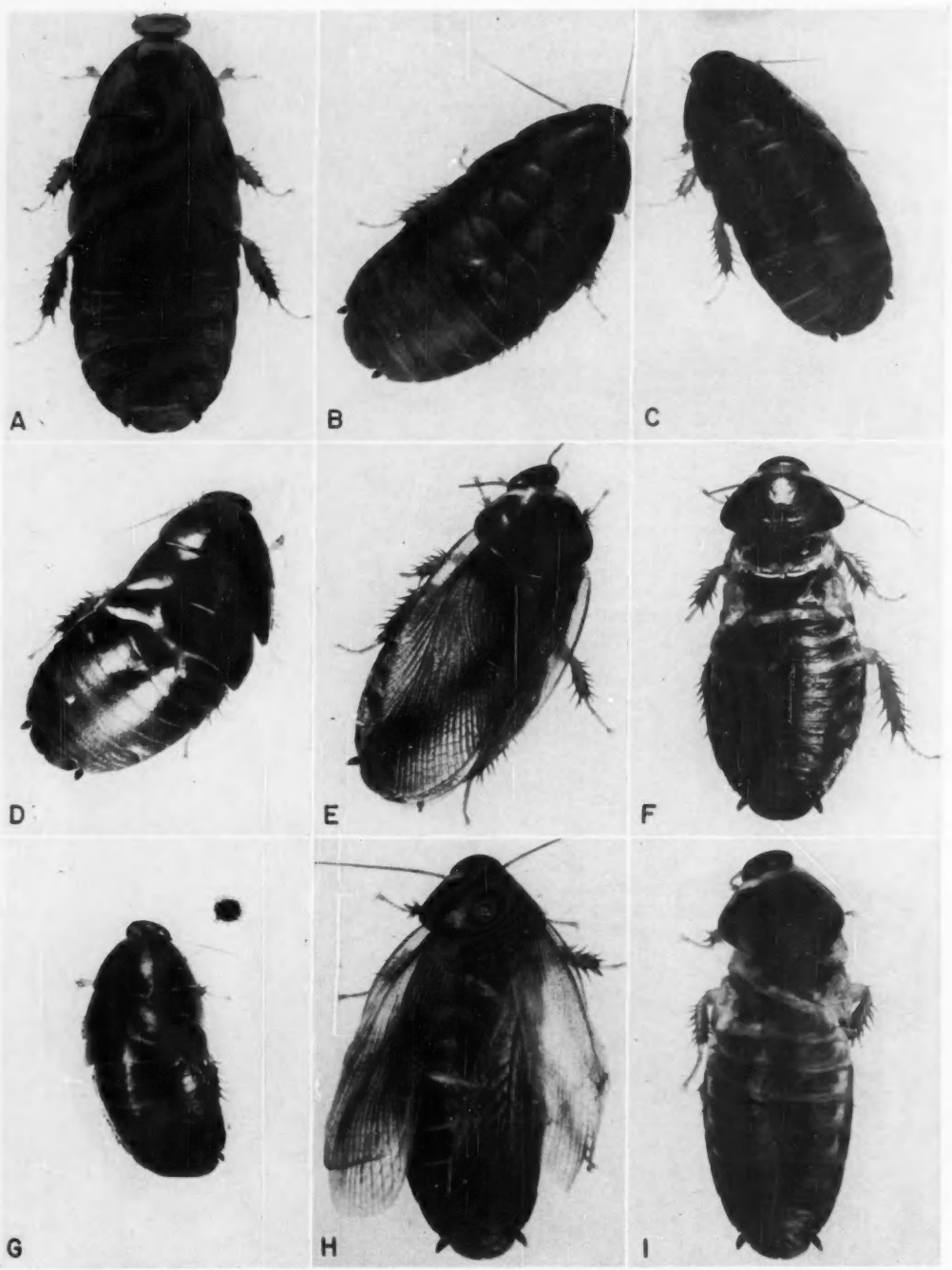

FIG. 4.—*Pycnoscelus surinamensis*, parthenogenetic strain from Florida. A.—Normal nymph. B-I.—Offspring of parthenogenetic-strain females mated to males of the bisexual strain. B, C, D, G, nymphs with aberrant tergites. E, adult female resulting from nymph in D; F shows this same adult female after wings were removed. H, adult female resulting from nymph in G, and I is this same female after wings were removed.

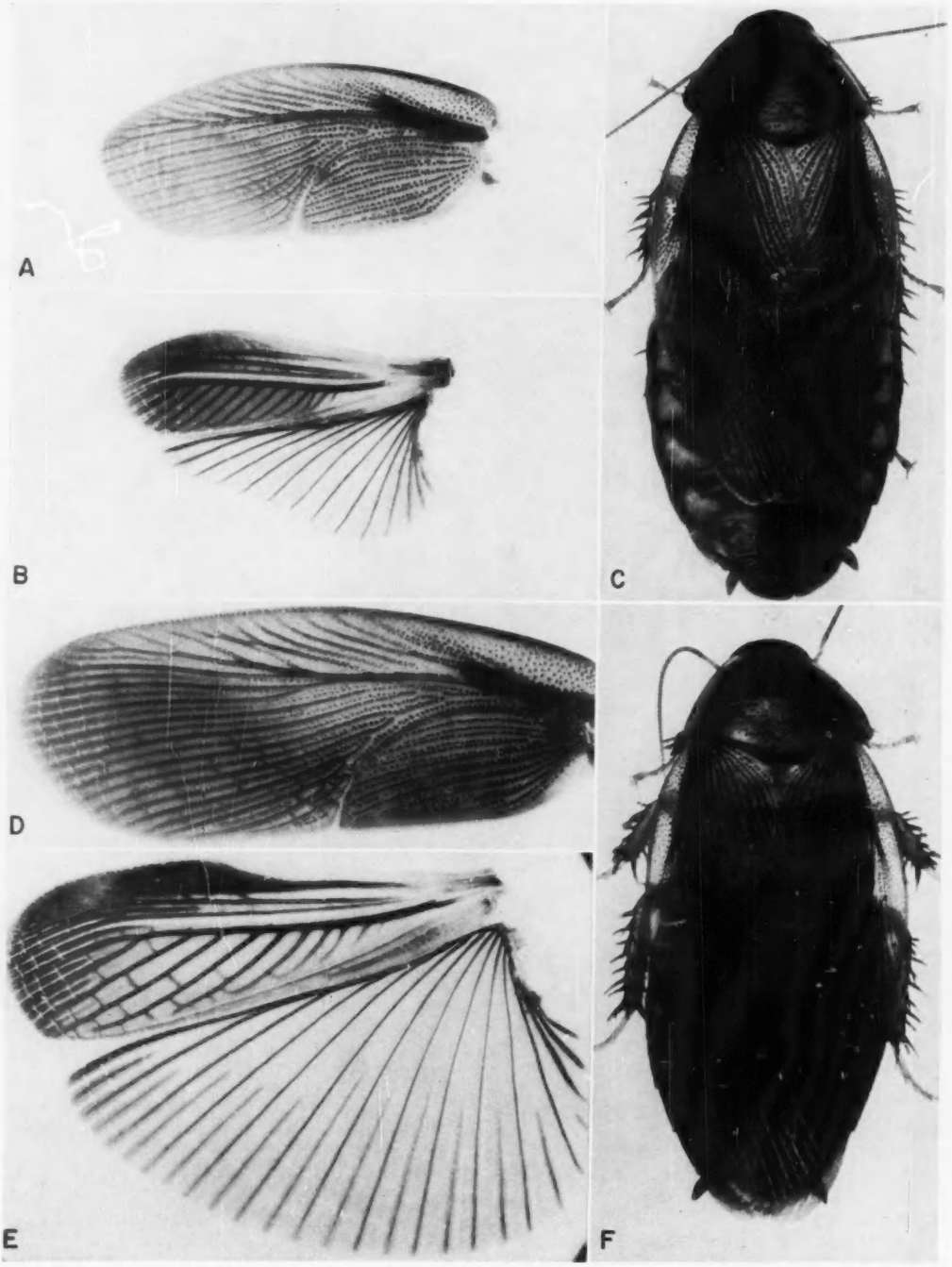

FIG. 5.—Gross morphological differences between two strains of *Pycnoscelus surinamensis*. A-C, bisexual strain from Hawaii (A, tegmen; B, wing; C, adult female. D-F, parthenogenetic strain from Florida (D, tegmen; E, wing; F, adult female).

Table 4.—Morphological differences between females of the bisexual and the parthenogenetic strains of *Pycnoscelus surinamensis*.[a]

Characters	Bisexual		Parthenogenetic		Significance of differences[b]	
	Mean ± S.E.	N	Mean ± S.E.	N	t	P
No. of rows of punctations across anal field of tegmen	16.7 ± 0.18 (14–19)	40	19.0 ± 0.15 (18–21)	40	9.60	<0.001
No. of cubital sectors in wing	12.7 ± 0.21 (10–15)	40	11.2 ± 0.14 (10–13)	38	5.71	<0.001
No. of veins in anal fan of wing	14.0 ± 0.14 (12–16)	40	15.5 ± 0.08 (15–16)	38	9.38	<0.001
Width of head	3.01 ± 0.031 (2.68–3.12)	20	3.17 ± 0.025 (2.92–3.36)	20	4.17	<0.001
Interocular distance	1.40 ± 0.026 (1.18–1.54)	20	1.20 ± 0.016 (1.10–1.34)	20	6.60	<0.001
Interocellar distance	1.18 ± 0.013 (1.08–1.26)	20	1.18 ± 0.015 (1.10–1.34)	20	0	>0.9
Distance from eye to ocellus	0.11 ± 0.005 (0.07–0.17)	20	0.04 ± 0.002 (0.03–0.07)	20	12.43	<0.001
Length of pronotum	4.56 ± 0.056 (4.1–5.0)	20	5.07 ± 0.050 (4.6–5.4)	20	6.72	<0.001
Width of pronotum	6.15 ± 0.017 (5.7–6.5)	20	6.85 ± 0.018 (6.2–7.3)	20	28.23	<0.001
Length of tegmen	12.9 ± 0.23 (10.8–14.5)	20	17.0 ± 0.14 (15.0–18.0)	20	15.05	<0.001
Width of tegmen	5.4 ± 0.09 (4.3–5.8)	20	6.3 ± 0.05 (6.0–6.7)	20	8.53	<0.001
Distance from plical furrow to apex of tegmen	6.2 ± 0.04 (5.0–7.3)	20	9.1 ± 0.13 (7.0–9.7)	20	20.98	<0.001
Length of wing	10.6 ± 0.21 (8.7–11.9)	20	14.8 ± 0.09 (14.0–15.3)	19	18.23	<0.001
Length of cercus	0.98 ± 0.014 (0.90–1.13)	20	1.06 ± 0.010 (1.00–1.16)	20	4.53	<0.001
Overall length	18.5 ± 0.42 (15.0–22.0)	20	21.2 ± 0.20 (19.8–23.0)	20	5.77	<0.001

[a] N = number of observations, S.E. = standard error; extreme values are given in parentheses; linear measurements are in millimeters.
[b] The probability of obtaining the observed differences between means by chance.

museum specimens this character may be more difficult to discern because the abdomens have shrunk. In living specimens the length of the abdomen in relation to the tegmina may vary depending on whether or not the female is pregnant, because the abdomen may be extended by the contained oötheca; this

Table 5.—Differences in ratios of linear measurements between females of the parthenogenetic and the bisexual strains of *Pycnoscelus surinamensis*.[a]

Character ratios	Mean values ± standard errors		Significance of differences[b]	
	Bisexual	Parthenogenetic	t	P
Pronotum, length : width	0.742 ± 0.0068	0.740 ± 0.0063	0.214	>0.8
Tegmen, length : width	2.395 ± 0.0125	2.683 ± 0.0184	12.96	<0.001
Tegmen length : pronotum length	2.832 ± 0.0301	3.351 ± 0.0331	11.61	<0.001
Tegmen length : distance from plical furrow to apex of tegmen	2.086 ± 0.0188	1.875 ± 0.0203	7.63	<0.001
Interocular distance : width of head	0.467 ± 0.0063	0.378 ± 0.0044	11.21	<0.001
Interocellar distance : interocular distance	0.842 ± 0.0095	0.984 ± 0.0100	10.34	<0.001

[a] Each mean value is based on measurements of 20 specimens.
[b] The probability of obtaining the observed differences between means by chance.

extended condition may also occur in museum specimens if the female does not abort her egg case at death.

The wings of the two forms differ in shape and venation (fig. 5, table 4). The margin of the anal fan of the parthenogenetic female curves convexly from the body to the plical furrow. But the margin of the anal fan of the bisexual female develops a slightly concave inflexion about midway from the body to the plical furrow. There are significantly more veins in the anal fan of the parthenogenetic female than in that of the bisexual female. However, despite its smaller size, the wing of the bisexual female has a significantly larger number of cubital sectors than that of the parthenogenetic. Intercalary veins are prominent in the wing of the parthenogenetic strain but less so, or are even wanting, in the wing of the bisexual female.

A significant difference was found between the numbers of rows of punctations across the anal field of the tegmina of the Floridian parthenogenetic female and the Hawaiian bisexual female (table 4); however, this character was extremely variable among museum specimens examined from other areas.

In parthenogenetic females the ocelli practically touch the eyes (fig. 6, top row), whereas there is usually a distinct space between eye and ocellus in the bisexual female (fig. 6, bottom row, table 4).

Museum Specimens.—The geographic distribution of the two forms of *Pycnoscelus surinamensis* can only be determined precisely by studying the reproductive behavior of samples from many areas. Because of overlapping morphological characters, museum specimens are difficult to identify exactly. The characters in both strains that show the least amount of overlap are tegmen length, distance from eye to ocellus, wing length, and tegmen width. Only the first two are readily determined in dried specimens, because the dried tegmina tend to curl around the narrow axis, making the width difficult to measure, and the wing is generally inaccessible.

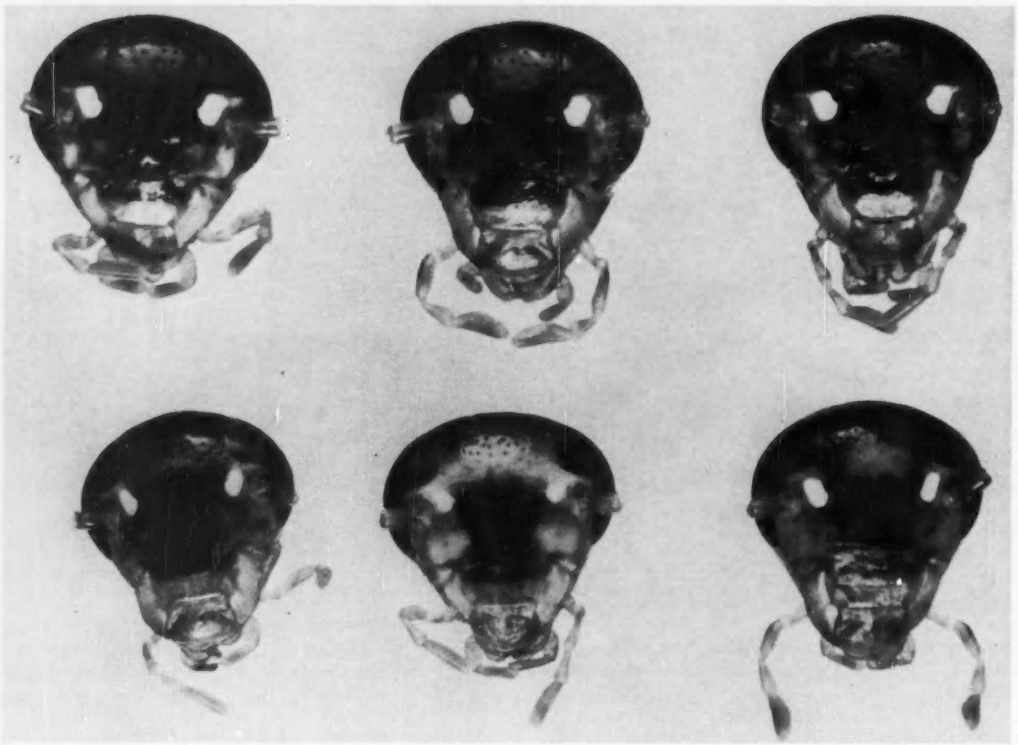

FIG. 6.—Heads of *Pycnoscelus surinamensis*. Top row: parthenogenetic strain from Florida. Bottom row: bisexual strain from Hawaii.

We have examined specimens from many different areas. It has not been possible to assign all of these definitely to either of the two strains we have described. However, we could tentatively decide from differences in tegmen length and the distance from eye to ocellus whether some of these might be one or the other of the two forms. Certain other specimens were anomalous in having a tegmen character of one strain combined with the eye-to-ocellus charac-

ter of the other. This suggests that we may not yet have determined the range of variation in these strains.

Examination of 18 females of *Pycnoscelus* from Hawaii confirmed the presence of both strains in that area. Most were the bisexual form. Of seven tentatively thought to be parthenogenetic on the basis of tegmen length, three showed a greater spacing between the eye and ocellus than the mean value for the bisexual form. In the other four specimens the eye-to-ocellus character fell well within the range observed in the Floridian parthenogenetic strain.

The measurements of five of nine females from Johnston Island fell in the range for the Hawaiian bisexual strain. The tegmina of the other four were just slightly longer than the maximum of our Hawaiian series.

We examined too few specimens from other Pacific Islands to warrant specific comment. However, most of the specimens from Eniwetok, Samoa, and Tonga had the tegminal character of the Floridian *P. surinamensis* and the eye-to-ocellus character of the Hawaiian bisexual form.

Among 25 females collected in Australia, mostly from Queensland, 16 had the long tegmina of the parthenogenetic strain and 9 had shorter tegmina that fell within the range of variation observed in the Hawaiian bisexual strain. Except for six females, which had a greater distance between eye and ocellus than the maximum previously observed in the parthenogenetic form, the 10 other females with long tegmina also displayed the eye-to-ocellus character of the Floridian females. It is interesting that although the bisexual female is apparently present in Australia, there were no males from Australia with the 24 females in the Bishop Museum collection; Dr. Gurney (1959) informed us that there are no males from Australia in the United States National Museum collection. Dr. Princis (1959) has also informed us that, as far as he is aware, no males have been hitherto recorded from Australia.

Specimens from southeast Asia and Indonesia could be assigned to both strains using the tegminal and eye-to-ocellus characters. This was not unexpected, as the bisexual form of *Pycnoscelus* has been reported to be endemic to this area.

The four African specimens we examined were similar to the Floridian strain, but the space between the eye and ocellus was wider.

The Mexican specimens were interesting in that 3 of the 18 had tegmina as short as the bisexual strain although they were not usually small individuals. The eye-to-ocellus character of only one of these also fell in the range for the bisexual form. So far as we know, no males of *Pycnoscelus* have ever been collected in nature in Mexico or elsewhere in the New World, with the exception of one reported from the New York Zoological Society (Davis 1919) and one from the New York Botanical Garden (Rehn 1945). None of the 15 females with long tegmina had a wider eye-to-ocellus distance than we have found in Floridian specimens; these undoubtedly belong to the parthenogenetic strain.

We examined a very few specimens (number indicated in parentheses) from each of the following continental New World countries: Brazil (3), Costa Rica (1), Guatemala (3), Honduras (3), Panama (4), El Salvador (2), and Venezuela (1); and the following West Indian islands: Cuba (4), Haiti (3), Jamaica (1), Puerto Rico (2) and St. Croix (1). All were females, and, except for one small individual from Cuba, the tegmina of all specimens fell within the range observed in the Floridian strain. However, the eye-to-ocellus distance of 14 of the 28 specimens fell in the range of the bisexual form.

Where the parallel characters of long tegmen and short eye-to-ocellus distance occur in the same population, we would assume that these females are parthenogenetic; conversely, when a wide eye-to-ocellus distance occurs with shorter tegmina, we would expect the females to be the bisexual strain. When these characters fail, we have at present no morphological basis for assigning the individuals to either strain. In the absence of biological observations, morphological characters in the present state of our knowledge are too variable to insure absolute identification of specimens from widely separated geographical areas. Obviously, much more attention must be paid to the behavior of living material if we are to arrive at a more nearly correct interpretation of the *surinamensis* complex.

DISCUSSION

Although facultative parthenogenesis may occur in at least five species of cockroaches (Roth and Willis 1956), obligatory parthenogenesis as exhibited by *Pycnoscelus surinamensis* in the New World appears to be unique among the Blattaria.

The results of this study show that the Hawaiian bisexual form of *surinamensis* differs noticeably from the parthenogenetic *surinamensis* from Florida. Besides the morphological differences shown in tables 4 and 5 and figure 5, the following physiological differences were noted: (1) the fertility of normally parthenogenetic females was reduced when they were mated to Hawaiian bisexual males; (2) the offspring from these interstrain matings were all females; which later reproduced parthenogenetically; (3) the bisexual strain, however, reproduced normally only when mated; virgin females of this strain did not ordinarily retract the oötheca into the brood sac; only one embryo of a bisexual female developed parthenogenetically, and it did not mature.

Although it is possible that two species are involved in this *Pycnoscelus surinamensis* complex and that the Hawaiian bisexual form may be a different species, perhaps undescribed, we have decided to consider the two forms as the same species in this paper. J. A. G. Rehn (personal communication), after reading our manuscript, has written us that ". . . no adequate study has been made of the external morphology of an extensive representation of

surinamensis from a broad range of the species' known distribution to clearly determine from these, and not from several experimental colonies, whether there is a warranted correlation of external morphological features with the demonstrated bisexual or parthenogenetic conditions. If we didn't have males from the areas mentioned above [Burma and Indonesia] and where the more usual type of female is clearly the dominant one, the picture would be different." Rehn further commented that we ". . . are dealing with a single species which is parthenogenetic over much of its area, but is bisexual in some places, as in Burma and Indonesia, from which areas we have males taken at quite a few localities. Unfortunately your breeding work was based almost, or quite, entirely on Hawaiian and Floridian material . . . . As to the morphological differences you give between the two, and I have read fully what you say about museum material, I think they are fully covered in a series of clearly *surinamensis* from many localities such as the one in our determined series, to say nothing of the scores of specimens from many localities in our [undetermined] series . . . I think I can say warrantedly you would find all the differences given by you in a series of females presumably all parthenogenetic, from the tropical and sub-tropical parts of the world. I frankly do not think your statistics would stand up under such a check, made visually and mensurably. What you need is not laboratory data, but data from broader feral or subferal populations from a number of localities. The same morphological tendencies happen regularly in other orthopteroid groups . . ."

Rehn may be correct in his interpretation, but the fact that, in a large series of specimens from widely separated geographical areas, the morphological characters for separating the parthenogenetic and bisexual strains may break down does not invalidate the possibility that sibling species (Mayr 1942; Mayr et al. 1953) are involved. As Mayr (1957) points out, sibling species ". . . form another decisive argument against defining species on a primarily morphological basis." And he further states that biological distinctness is primary and morphological differences secondary; the morphological concept is abandoned when it comes in conflict with biological data. White (1954) stated that ". . . it is illogical to recognize parthenogenetic and bisexual 'races' of the same species (irrespective of the morphological resemblances between these genotypes); such forms are better regarded as sibling species if they are indistinguishable or barely distinguishable by ordinary taxonomic criteria."

If one tries to apply the biologic species concept to the strains of *Pycnoscelus surinamensis*, the parthenogenetic and bisexual strains, at first glance, might be considered different species. Both strains occur in Hawaii but there is apparently little or no interbreeding between the bisexual male and females of the parthenogenetic strain. Even if the parthenogenetic female mated with a bisexual male, the offspring, on the basis of our experiments, would be all females which reproduce parthenogenetically and which are morphologically typical parthenogenetic females. Thus there is no gene flow between these two strains and they can not contribute to a common gene pool, even if they are sympatric. Difficulty may arise in the possibility that the parthenogenetic strain may have no males associated with it and is obligatorily parthenogenetic. This is certainly true of *P. surinamensis* in the New World, where the form may be considered asexual. The problem in applying the modern species concepts to parthenogenetic forms lies in the fact that "In truly asexual organisms there are no 'populations' in the sense in which this term exists in sexual species nor can 'reproductive isolation' be tested." (Mayr 1957). A biologic study of *Pycnoscelus surinamensis* obtained from the Indomalayan region (i.e., the area in which the genus is endemic) would certainly aid in solving this problem. It is possible that in Burma and Indonesia the bisexual strain, if it exists, may well have a male that is different from the male of the Hawaiian bisexual strain.

Matthey (1948) claimed to have studied the chromosomes of both the parthenogenetic and alleged bisexual "varieties" of *P. surinamensis*. He reared male and female adults from nymphs collected in Kuala-Lumpur, Malaya. One of the females reproduced parthenogenetically, but, at the time of his writing, Matthey had not determined the sex of the offspring and stated that they were probably females. Matthey concluded that either (1) the specimens received from Malaya included individuals of two "varieties", one bisexual and the other parthenogenetic, or (2) there is only one "race": if the females are not fertilized, they produce females; if they are fertilized, the offspring will be bisexual. Matthey favored the second hypothesis, but, unfortunately, as far as we know, he did not follow through to show that the parthenogenetic female will produce both sexes if mated. Ideally, to determine whether a male is associated with the parthenogenetic strain, it should be shown that known parthenogenetic *P. surinamensis* females are capable of being fertilized by a male and producing bisexual young with no reduction in fertility. To the best of our knowledge this has never been demonstrated, nor has a known female of the parthenogenetic strain ever been reported as giving birth to young of both sexes.

Roeser (1940), who studied the biology of *Pycnoscelus surinamensis* obtained from the Zoo and Botanical Garden in Berlin, Germany, concluded that two "breeds" existed, a small "Minor" form (18.9-21.0 mm. long) and a larger "Major" form (21.9-24.9 mm. long). He also noted differences in color between these two forms. Apparently both forms reproduced parthenogenetically. Roeser made no comment about the relative length of the tegmina in relation to the abdomen, but from his photograph (p. 193) the tegmina of the "Minor" female reach almost to the end of the abdomen. Suomalainen (1945)

found that females of the "Major" and "Minor" strains both have 36 chromosomes. The occurrence of a small "Minor" form of *P. surinamensis* somewhat limits the use of absolute tegmen length as a character difference between the Hawaiian bisexual female and the parthenogenetic female; however, a ratio of tegmen length to overall body length might be more useful, although, as we have pointed out, the abdomen tends to shrink in museum specimens and abdomen length may also vary depending on whether or not an oötheca is contained in the uterus.

The fact that Roeser (1940) found two apparently morphologically distinct forms of a parthenogenetic strain of *P. surinamensis* is of special interest in an attempt to explain the variation found among museum specimens from various geographical areas. Once a female capable of reproducing parthenogenetically has been introduced into an area devoid of males, there would be relatively little chance for further variation in the morphological characters of her offspring, except through mutation which might or might not be expressed in the phenotype. It is generally accepted that parthenogenesis in existing forms is a secondary method of reproduction, having arisen from the bisexual condition. The parthenogenetic strain of *P. surinamensis* undoubtedly evolved from a bisexual form (perhaps the Hawaiian bisexual strain or one very similar to it). It is conceivable that the existing variable presumably parthenogenetic "populations" are descendents of a number of variable forms that evolved from a common ancestor. The morphological characters typical of parthenogenetic females in a given geographical area would depend on the form of female or females originally introduced into that area.

If two sibling species are involved, then there are plausible explanations for our breeding results. One of the sexual isolating mechanisms between species is a lowered fertility when different species mate. Viable hybrids may not be produced ". . . either because fertilization is prevented (inability of the spermatozoa to reach or to penetrate the eggs) or because after fertilization there are cytogenetic disturbances . . ." (Mayr 1942). Possibly the eggs which hatched from the mated parthenogenetic females of *P. surinamensis* in our experiments were never fertilized, and therefore the resulting, exclusively female offspring were morphologically and physiologically typical of the parthenogenetic female parent. Perhaps fertilization actually prevented development of some of the eggs in parthenogenetic females; this could account for the lower rate of fertility among these mated females.

Since it is possible that the Hawaiian bisexual strain is a different species from the parthenogenetic strain, one may speculate whether it represents a new species or whether it has already been described and the name placed in synonymy with *P. surinamensis*. If the two strains eventually are shown to be distinct species, the Hawaiian bisexual form will have to be renamed. Linnaeus (1767) described *P. surinamensis* from specimens obtained from Surinam, South America. Since males do not occur in nature in the New World, it is evident that Linnaeus based his description on the parthenogenetic strain. One noticeable difference between living bisexual and parthenogenetic forms is the length of the tegmina in relation to the length of the abdomen (cf. figures 5C and 5F); this difference in relative wing length may be less discernible in pinned specimens. However, it is interesting to note that Burmeister (1839) separated *Blatta indica* Fabricius from *Panchlora surinamensis* (L.) because *indica* (from the East Indies), which was constantly smaller and proportionately wider, had wings that were scarcely longer than the dried abdomen and only about half as long as those of *P. surinamensis*. But Brunner (1865, pp. 278-280) included *indica* as a variety in the synonymy of *P. surinamensis*:

> *Varietas minor, elytris abbreviatis:*
> *Blatta indica* Fabr.
> *? Blatta corticum* Serv.
> *Panchlora indica* Burm.

Brunner (1865, p. 279) commented on *P. surinamensis*: "Cette espèce est repandue dans tous les pays chauds, où elle s'est domestiquée, et diffère par la même de tous ses congénères. Sa taille est variable, ce qui a engageé M. Burmeister à en former deux espèces, l'une orientale, l'autre occidentale. J'ai un grand nombre d'individus devant moi, qui représentent tous les passages entre ces prétendues espèces. La variété à longs élytres provient indistinctement de l'orient et de l'occident, tandis que les individus les plus petits, à élytres raccourcis, ne se trouvent en effet que parmi ceux d'origine indienne."

From this it would appear that in 1865 at least two forms of *Pycnoscelus* were being considered as *P. surinamensis*; Fabricius' (1775) *Blatta indica* might have been the same as the Hawaiian bisexual form, although his description (p. 272) is insufficient to distinguish it from the parthenogenetic strain. Dr. K. Princis has been kind enough to compare females of the bisexual Hawaiian material with the type specimen of *Blatta indica* Fab., which is in the Fabrician collection in the Copenhagen Museum. He wrote us (1959) that "They are not at all identical. [The] Fabrician species is clearly based on the parthenogenetic form of *Pycnoscelus surinamensis*." As for *Blatta corticum* Serv. (type locality Mauritius, and listed above by Brunner as having abbreviated elytra), Princis commented that "It seems to me that this is a valid species and has nothing to do with *surinamensis*. I have namely found a topotypic male in the collection of the Strasbourg Museum and it differs widely from *surinamensis*. It is quite possible that this is the true *corticum* the type of which is lost."

It is quite possible that the male of the bisexual form from Hawaii has already been described and is now considered a synonym of *Pycnoscelus surinamensis* (i.e., the parthenogenetic strain). *Panchlora celebesa* Walker (described from Celebes; Walker

1868) and *Panchlora occipitalis* Walker (described from Bombay; Walker, 1871), based on males, are now considered to be synonyms of *P. surinamensis*. Ragge (1959) has compared our males of the Hawaiian bisexual strain with type specimens of *P. celebesa* and *P. occipitalis* in the British Museum of Natural History. He stated that the Hawaiian males ". . . are *Pycnoscelus occipitalis* (Walker). There is very close agreement with the holotype in all the characters which Gurney mentions, and what is visible of the genitalia is almost very similar. *P. celebesa* (Walker) is smaller, and its pronotum differs in colour." Gurney (1959) had suggested that males be compared as to general coloration, especially of the pronotum and tegmen; distances between compound eyes and between ocelli; and punctation and shape of the pronotum.

Gurney (1959) has stated "In view of the frequency with which *Pycnoscelus* is transported in commerce, and the large percentage of Hawaiian insects (at least the more domestic and lowland forms) which have come from elsewhere, the whole story of what constitutes the various species of *Pycnoscelus* and how they are distributed may yet require considerable investigation."

Rehn (1959) regards the Hawaiian colony of *Pycnoscelus surinamensis* as having been brought, within the last century or so, from Indomalaya either directly or indirectly. Rehn further believes that the Floridian colony of *P. surinamensis* did not come directly from Indomalaya, probably not even indirectly, but doubtless came from the West Indies to which it was probably carried in African slave ships. According to Rehn, *P. surinamensis* probably reached Africa (and Madagascar) centuries ago in Arab dhows, which maintained Indian Ocean trade long before the first Portugese entered it.

It is curious that only the parthenogenetic strain of *Pycnoscelus surinamensis* has been introduced (or has become established) in the New World. However, a form that does not depend on the male for reproduction has a greater opportunity for establishing itself when introduced into a new, climatically favorable area than an obligate bisexual species. Thus, the parthenogenetic strain of *P. surinamensis* can become established following introduction of a single nymph or an unmated adult female. With an obligate bisexual strain of *Pycnoscelus* the female would require mating prior to introduction or both sexes would have to be introduced, survive, and remain in close enough proximity to find each other and mate when sexually mature.

Perhaps the ecological requirements of the two strains of *Pycnoscelus surinamensis* under discussion differ, with the result that the parthenogenetic strain is more likely to be introduced into new areas because of the niche it occupies in nature. In the two shipments we received from Hawaii, in 1958, practically all specimens were of the bisexual strain; there were only 2 parthenogenetic females among 100 bisexual females in one shipment, and none in the second. This high ratio of bisexual to parthenogenetic forms may indicate that each strain tends to inhabit a separate niche in nature, although there may be some overlapping. It is especially interesting that one strain of *P. surinamensis* has evolved a method of maintaining itself parthenogenetically, whereas the virgin female of the Hawaiian bisexual form is virtually unable to retract the oötheca and in the laboratory is incapable of reproducing parthenogenetically. The ability of the virgin female of false ovoviviparous cockroaches to place the oötheca in the brood sac is a prerequisite to the evolution of parthenogenesis in the Blattaria.

Hawaii offers an excellent opportunity for an ecological study of these two strains—a study which should answer many of the questions raised in this discussion.

In 1926 and 1927 *Pycnoscelus surinamensis* was shown to be the intermediate host of the chicken eyeworm *Oxyspirura mansoni*, by Fielding in Australia, Kobayashi in Formosa, and Sanders in Florida (see review by Roth and Willis 1957). Undoubtedly Sanders, at least, was working with the parthenogenetic strain, which is the only form that occurs in Florida. The question may be raised as to whether or not the bisexual strain of *Pycnoscelus* could act as a vector of this nematode.

Schwabe has published on *Pycnoscelus surinamensis* as the intermediate host of *O. mansoni* in Hawaii. In one paper (1949) he stated that the tegmina of female *P. surinamensis* do not completely cover the abdomen; in a later publication (1950) he illustrated (p. 181, fig. 3) an adult female *Pycnoscelus* which appears to be, from its short tegmina, a female of the bisexual strain. He also mentions males of this species; if the illustrated female represents the form Schwabe used as hosts of the chicken eyeworm in his experimental work, then the bisexual strain of *Pycnoscelus* in Hawaii also serves as an intermediate host of *Oxyspirura mansoni*.

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Biology of *Hyposoter exiguae* (Hymenoptera: Ichneumonidae), A Parasite of Lepidopterous Larvae[1]

BENJAMIN PUTTLER

Entomology Research Division, Agricultural Research Service,
U. S. Department of Agriculture

ABSTRACT

Hyposoter exiguae (Viereck), a Nearctic ichaeumonid, is a solitary endoparasite of the larvae of several species of Lepidoptera. It prefers to oviposit in late first- and second-instar larvae and emerges from the third. Three larval instars have been observed, the last leaving the host body to spin its cocoon. At 72° to 76° F. the insect spends 2 days in the egg stage, 7 days as a larva, and 7 to 8 days in the cocoon. Females mate only once, but males mate with more than one female. Mated females produce about equal numbers of both sexes; unmated females produce males only. Temperature was found to be the main factor influencing the life cycle and longevity of the parasite. Developmental time at 60° to 80° F. ranged from 33.8 to 14.6 days. Longevity at 40° to 80° F. ranged from 50.5 to 6.5 days. Percent emergence from cocoons was also related to temperature. Those held longest at 40° F. prior to removal to 72° to 76° F. showed emergence of only 20% as compared with 100% for those held for the shortest period. *Spilochalcis albifrons* (Walsh) was the most frequent hyperparasite reared. It oviposits in the cocoon of *H. exiguae*, and its life cycle requires 23 days at 72° to 76° F.

Hyposoter exiguae (Viereck) is a solitary endoparasite of lepidopterous larvae, most of which belong to the family Noctuidae (Puttler and van den Bosch 1959). Since its hosts represent a large group of pest species attacking a wide variety of agricultural crops, knowledge of the factors that might contribute to their natural control may be of considerable economic importance. The biological investigations reported herein supply some of this information.

HISTORY

Hyposoter exiguae was described originally by Viereck (1912) under the name *Campoplex (Hypothereutus) exiguae*. The type specimens were reared from larvae of *Laphygma exigua* (Hbn.) collected at Davis and Santa Ana, California. Viereck

[1] These studies were conducted in cooperation with the Department of Biological Control, University of California, Riverside. R. van den Bosch and E. I. Schlinger, of the University of California Citrus Experiment Station, made suggestions in the preparation of this manuscript. B. D. Burks, of the Entomology Research Division, Agricultural Research Service, U.S.D.A., identified the hyperparasites *Spilochalcis albifrons* (Walsh) and *Certasmicra* n. sp.

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(1926) also described *Campoplex (Ameloctonus) eurythemae,* reared from larvae of *Colias philodice eurytheme* Bdv. collected at Saskatchewan, Canada. *Campoplex (A.) eurythemae* has since been placed in synonymy with *H. exiguae.*

Very few references to *H. exiguae* are found in the literature. The first report of the parasite subsequent to its description was by Swezey (1927). He referred to its accidental introduction into Hawaii from California and its establishment and rapid spread throughout the Islands. He also noted its value as an armyworm parasite. Later Michelbacher and Essig (1938) and Wilcox et al. (1956) reported it to be a major parasite of the tomato fruitworm, *Heliothis zea* (Boddie), in California.

DISTRIBUTION

This species is restricted to the Nearctic region, and has been recorded from the following areas in the United States: Arizona (Swezey 1927); California, Colorado, New York, South Carolina, District of Columbia (Muesebeck et al. 1951); Utah (Wilcox et al. 1956); the Hawaiian Islands (Swezey 1927); and Saskatchewan, Canada (Muesebeck et al. 1951). The author has also seen two specimens from the Mexicali Valley, Mexico (reared by E. Nye, November 1957).

HOSTS

Hyposoter exiguae has a wide host range within the Lepidoptera. The author has reared it from the following hosts in southern California: *Heliothis zea* (Boddie), *Prodenia praefica* Grote, *Prodenia ornithogalli* Guen., *Peridroma saucia* (Hbn.), *Laphygma exigua* (Hbn.), *Pseudaletia unipuncta* (Haw.), *Trichoplusia ni* (Hbn.), *Autographa californica* (Speyer), *Catabena esula* (Druce), all in the Noctuidae; *Protoparce quinquemaculata* (Haw.), Sphingidae; and *Vanessa cardui* (L.), Nymphalidae. In the laboratory it has completed its development on two other noctuids, *Laphygma frugiperda* (J. E. Smith) and *Autographa biloba* (Steph.). Additional host records include *Colias philodice eurytheme* (Bdv.) (Muesebeck et al. 1951) and *Anthocaris genutia* (F.) (Krombein et al. 1958), both Pieridae; *Laphygma exempta* (Walk.) and *Plusia chalcites* (Esp.), Noctuidae; and *Scotorythra paratactis* Meyrick (Swezey 1927) and *Scotorythra paludicola* (Butler), both Geometridae (Fullaway 1947).

It is interesting that *Laphygma exigua,* although the host from which *Hyposoter exiguae* was originally described, was found to exhibit a partial immunity to the parasite (Puttler and van den Bosch 1959); this was not observed in any other hosts from which the author obtained life-history data or from other hosts recorded in the literature.

In California *H. exiguae* has been reared from its hosts most commonly in association with the following plants: Tomato, alfalfa, lettuce, cabbage, cotton, and various grasses.

LABORATORY REARING

In order to carry out the biological studies on *Hyposoter exiguae,* it was necessary to develop an efficient method of rearing both the parasite and a suitable host species. Of several possible hosts, *Peridroma saucia* (the variegated cutworm) was chosen for the following reasons: (1) it could easily be reared in small containers (2) it was attacked readily by *H. exiguae* in the laboratory, (3) the eggs were laid in masses, (4) it was essentially noncannibalistic, (5) it showed little evidence of disease in its early instars, and (6) adult moths were easily collected at lights, insuring a steady supply of host material.

Female moths collected at lights were brought into the laboratory and placed in gallon jars lined with wax paper. The first eggs were usually deposited within the first 48 hours and oviposition continued until the females died, after about 5 days. The egg masses were periodically removed and placed in round, half-pint ice-cream cartons with petri dish covers. On hatching, the larvae were fed on alfalfa bouquets, which were replenished daily or as needed.

When the host larvae had reached the size preferred for attack by *H. exiguae* (late first and second instars), a single female was placed in a carton containing 25 to 50 host larvae, and allowed to oviposit for 24 hours before being removed. Under these conditions 40% to 80% parasitization usually was obtained. Two days after the parasite had been removed, the host larvae were separated into smaller groups and allowed to complete their development. Emerging parasite adults were used for immediate propagation or held at low temperatures (40° or 50° F.) until needed.

Other hosts were obtained in the same manner as *P. saucia* or by means of eggs collected in the field. They were reared under the same conditions, with the exception of *Vanessa cardui* which was reared on *Malva,* and *Catabena esula* on *Lantana.* All data and observations are derived from tests in which *P. saucia* served as a host. Larvae were reared at 72° to 76° F. and 30% to 50% RH, unless otherwise stated.

MATING

Hyposoter exiguae mated readily in the laboratory at 72° to 76° F. and 30% to 50% RH. Matings were observed under the following conditions: (1) in shell vials, where one male and one female were placed together, (2) in the rearing units in which a male was placed with a female for 24 hours (the progeny in these units were of both sexes and mating must have taken place), and (3) in a cage 17 inches long, 17 inches wide, and 11 inches high, with a wood frame, glass top, and cloth-covered sides. Mating was confirmed by the presence of both sexes among the progeny reared from each kind of unit. Mating took place most readily in cages. Two males per female gave the best results. Indirect sunlight from a window with a western exposure also appeared to hasten mating.

Successful mating depended largely on the ability of the male to grasp the female. In shell vials the parasites often became highly excited, and this apparently interfered with the male's ability to grasp the female. In the cage, where the females were less active, males were able to make successful contact with much greater frequency.

Mating occurred between individuals of various ages, and most matings involved newly emerged adults with individuals that were up to 1 week old. Some individuals older than 1 week were observed to mate. No female mated more than once, but males frequently mated with more than one female. As indicated by 20 observed matings the actual time spent in copulation appeared unusually long, ranging from 5 to 25 minutes, with a mean of 10.6 minutes.

OVIPOSITION

Hyposoter exiguae females are capable of ovipositing immediately after they have completely emerged. Newly emerged females, when dissected, have been found to contain as many as 101 mature eggs in their oviducts. There does not appear to be any strong olfactory attraction to host larvae. Direct contact with the host is apparently the stimulus to oviposition, which takes place in less than a second in stationary host larvae. Should the host move while the parasite is attempting to oviposit, the parasite will search for another host. However, if in the process of searching she again comes in contact with the previously missed host, she may make another attempt.

The fecundity of *H. exiguae* was difficult to determine owing to the absence of sufficient host material over the entire life of the parasite. Data were obtained on the number of eggs laid by freshly emerged parasites when exposed to host larvae over a period of 24 hours at 72° to 76° F. and 30% to 50% RH. Two females, each with 50 host larvae, laid 47 and 52 eggs. Two other females, each with 25 host larvae, laid 25 and 29 eggs over the same period of time. Another female in the presence of 200 host larvae (50 during each 24-hour period) laid 111 eggs in 96 hours. Of this number 60 were laid during the first 24 hours. The parasite appears capable of laying the majority of its fully developed eggs during the first 24 hours providing sufficient hosts are available. The number of eggs laid was determined by dissection of the exposed host larvae.

H. exiguae females oviposit in first-, second-, and third-instar host larvae. Eggs laid in the first two instars complete their development and adults emerge from the third-instar hosts. Preference has been noted for the late first and the second instars. Early first instars are readily disturbed and either move or drop from the plant as the parasite approaches. Eggs deposited in third-instar hosts do not complete their development. Numerous dissections revealed that, though the eggs usually hatch, the first-instar parasite larva always becomes encapsulated by blood cells of the host, and the host is able to develop to maturity. Phagocytosis occurs rarely in first- and second-instar hosts. In addition to *Peridroma saucia*, phagocytosis was observed also in *Autographa californica* and *Prodenia praefica*, and possibly occurs in all other hosts.

DEVELOPMENT

The egg of *Hyposoter exiguae* is deposited free in the haemocoele, usually of the posterior part of the host's abdomen, and the larva is free-living in the body cavity. Three larval instars have been observed. Tothill (1933) found the same number in the related *H. pilosulus* (Provancher). He described the egg and larval stages of that species, and since the gross anatomies are similar no attempt is made to describe the various stages of *H. exiguae* at this time. *H. pilosulus* also exhibits other biological similarities with *H. exiguae* (Tothill 1922; Swaine et al. 1938). However, there are distinct differences between the two species. *H. exiguae* is multivoltine, prefers first and second instars for oviposition, and forms its cocoon after emerging from the host larva, whereas *H. pilosulus* is univoltine oviposits in second-, third-, and fourth-instar hosts, and forms its cocoon within the skin of the host.

The approximate times for the development of the immature stages of *H. exiguae* were determined by means of dissections made every 24 hours from the time of oviposition until adult emergence. All host larvae used for oviposition and development were of the late first and second instars.

At 72° to 76° F. and 30% to 50% RH, the egg, second instar, third instar, and prepupal stage each required 2 days, the first instar 3 days, and the pupal stage 5 to 6 days. Development from egg to adult therefore required 16 to 17 days.

After consuming the body contents of the host, the parasite larva breaks the host skin with its mandibles and emerges from the posterior end. It may remain in this position and form its cocoon or may move about for several minutes before settling down and spinning the cocoon. The cocoon is normally completed in 2 to 4 hours and is usually found with the cast skin of the host attached to it.

The cocoon is comprised of two layers. Upon emerging from the host the larva first spins an outer covering of grayish silk. It then forms an inner layer, which is tough and parchment-like, and dark brown in color. The cocoon is 6 to 7 mm. long and its greatest width 2 to 3 mm. It is usually whitish-gray or grayish-brown with two broken brown rings equidistant from the ends. These brown rings are formed where the outer layer is very thin and the inner parchment-like surface shows through.

The adult emerges by cutting a circular hole in the dorso-median tip of the cocoon. In the progeny of a mated female there are about equal numbers of males and females. Four mated females produced 62 females to 57 males. No tendency was observed for one sex to emerge before the other. Unmated females produce only male progeny.

FACTORS INFLUENCING LIFE CYCLE

Temperature is a prime factor influencing the life cycle of *Hyposoter exiguae*. To determine its effect, parasites were placed with host larvae for 24 hours at 72° to 76° F. and then transferred and held for development at 60°, 72° to 76°, or 80°. As indicated in table 1, there was no significant difference between the times required for development at 72° to 76° and 80°, but at 60° the period was about twice as long.

Table 1.—Development period of *Hyposoter exiguae* at different temperatures.

Temperature (° F.)	Relative humidity (%)	Number of insects	Development period (days)					
			Egg-cocoon		Cocoon-adult		Total	
			Range	Mean	Range	Mean	Range	Mean
60	30	15	20–24	20.3	13–15	13.5	31–38	33.8
72–76	30–50	100	8–10	9.1	7–8	7.2	15–18	16.3
80	40	20	8–10	8.9	5–7	5.7	14–16	14.6

The parasite was observed to complete its development only between 60° and 80° F. At 90° it formed a cocoon (7-8 days), but the adult did not emerge. Dissections revealed that the prepupa and pupa within the cocoon had desiccated. At this temperature the relative humidity, which was 10% to 20%, may have been the limiting factor, and a higher relative humidity might have resulted in some adult emergence. At 50° the host and parasite development appeared to be unsynchronized. Sometimes the parasite developed faster than the host, causing the host to die before it became large enough to support the complete development of the parasite larva, and sometimes the reverse situation occurred resulting in the death of the parasite larva and survival of the host. The cause of the parasite's death was not determined, but it was always in the second instar.

The age of the host larva at the time of oviposition was also found to influence the length of the parasite's life cycle. In table 2 it can be seen that an additional 5.7 days were required when oviposition occurred early in the first instar as compared to the late first and second instars. The longer time was spent in the egg and larval stages, since the cocoon-adult period was not appreciably different.

The results of limited studies on the development of *H. exiguae* on some of its other hosts are presented in table 3. It is readily seen that no appreciable differences exist at the temperatures under which the study was made.

ADULT LONGEVITY

Freshly emerged adults reared at 72° to 76° F. and 30% to 50% RH were fed honey for 15 minutes, and then lots of 10 to 40 individuals were exposed to temperature and relative humidity conditions as shown in table 4. Those held at 72° to 76° were exposed to 8 to 10 hours of light each day. Other lots were exposed to continuous light. Individuals held at 72° to 76° were held in the cage used for the mating tests and fed honey every other day. All other individuals were placed singly in shell vials with a drop of honey. Honey is essential for newly emerged adults; mortality was observed within 48 hours if food was not available. In the presence of a food source, temperature becomes a dominant factor, though its effect on longevity, as shown in table 4, may be related to the associated relative humidity. Additional observations indicated that females survive about 2 to 7 days longer than males, especially at the lower temperatures (40° to 60° F.).

Table 2.—Time of development of *Hyposoter exiguae* at 72° to 76° F. when ovipositing in hosts of two age groups.

Host age	Number of insects	Development period (days)					
		Egg-cocoon		Cocoon-adult		Total	
		Range	Mean	Range	Mean	Range	Mean
Early first instar	15	14–15	14.5	7–8	7.5	21–23	22.0
Late first and second instars	100	8–10	9.1	7–8	7.2	15–18	16.3

Table 4 shows that the parasite attains greater longevity as the temperature is decreased from 80° to 40° F. The mean longevity of 50.5 days at 40° indicates that it is fairly tolerant to cool temperatures. This may be one of the factors accounting for its occurrence in cool areas where it may overwinter as

Table 3.—Length of life cycle of *Hyposoter exiguae* in different hosts at 72° to 76° F. and 30% to 50% RH when oviposition occurred in late first- and second-instar larvae.

Host	Number of individuals	Mean development period (days)
Peridroma saucia	100	16.3
Autographa californica	42	16.3
Laphygma frugiperda	25	17.2
Prodenia praefica	20	18.0
Vanessa cardui	6	17.1
Catabena esula	6	17.6
Autographa biloba	2	16.5

an adult when hosts are not available. On the other hand, in warmer areas, such as southern California, many of its hosts are present throughout the year and continuous generations are produced. The author has never observed any form of diapause either in the field in southern California or in the laboratory.

In connection with the longevity study, observations were made on the viability of eggs laid by females held at 40°, 50°, and 60° F. for 50.5, 28.1, and 14.4 days, respectively. As noted earlier, *H. exiguae* females are capable of depositing mature eggs immediately after emergence, and it seemed possible that prolonged retention of eggs at low temperature might adversely affect their viability. The days of exposure to the above temperatures represent the mean longevity of adult females in table 4. After these periods adults were placed in half-pint ice-cream cartons at 72° to 76° F. and offered host larvae. Oviposition occurred readily with subsequent successful development of the parasite larvae. The number of mature eggs (as many as 101) found in the oviducts of freshly emerged female parasites was the same as that found in females held at the lower temperatures, indicating that prolonged exposure at these temperatures had no effect on their viability.

To determine the effect of 40° F. and 50% RH on the cocoons for various periods of time, 20 freshly formed cocoons were reared at 72° to 76° F. and 30% to 50% RH and placed at 40° F. Every 7 days 5 cocoons were removed and held at 72° to 76° and 30% to 50% RH for emergence. In table 5 it can be seen that the *H. exiguae* cocoons can tolerate temperatures as low as 40° for at least 28 days. Although no emergence occurred at this temperature during this test, two things became evident: (1) As the exposure increased, there was a slight decrease in the mean number of days required for emergence after being removed to 72° to 76°. This was apparently due to the parasites' slowly continuing development within the cocoon. (2) The total emergence decreased from 100% to 20% as the period of continuous exposure at 40° increased. It thus appears doubtful that much emergence would occur if the cocoons were held at this temperature for more than 28 days.

Table 4.—Longevity of adult *Hyposoter exiguae* at different temperatures and relative humidities.

Temperature (°F.)	Relative humidity (%)	Number of individuals	Longevity (days)	
			Range	Mean
80	40	10	5–9	6.5
72–76	30–50	40	4–16	9.5
60	30	20	8–23	14.4
50	60	20	3–49	28.1
40	50	20	4–82	50.5
25–35	70–80	10	13–35	23.2

Table 5.—Effect of 40° F. on the developing parasite in the cocoon and the subsequent emergence at 72° to 76° F.

Days at 40° F.[a]	Mean days to emergence	Percent emergence
7	5.6	100
14	5.0	100
21	4.3	60
28	4.0	20

[a] Five cocoons held for each period.

SUPERPARASITISM

Hyposoter exiguae females apparently cannot distinguish between parasitized and nonparasitized hosts. When 50 larvae each of *Peridroma saucia* and *Autographa californica* were exposed to one female *H. exiguae* for 24 hours, 42 of the former and 41 of the latter were parasitized and 10 and 4 of the respective hosts were superparasitized. The maximum number of eggs found within a single host was six. Most dissected superparasitized hosts contained only two eggs. Regardless of the number of eggs deposited, only one larva completed its development, apparently the first individual to hatch. The supernumerary eggs usually hatched, but the first-instar larvae soon became encapsulated and died. In some instances encapsulation of supernumerary eggs was observed. Disposal in this manner strongly suggests internecine action and that the dead eggs or young larvae are decomposed by phagocytic cells of the host. Superparasitism did not appear to have an adverse effect on the surviving parasite, since it eventually completed its development.

HYPERPARASITES

Two species of hyperparasites were reared from field-collected cocoons of *Hyposoter exiguae*. These were *Spilochalcis albifrons* (Walsh) and *Certasmicra* n. sp. Only one specimen of the latter species was reared, and its host was presumed to be *H. exiguae* parasitizing *Catabena esula*. *S. albifrons* was reared most frequently from *H. exiguae* cocoons collected on tomato.

Limited biological data were obtained on *S. albifrons*, with *H. exiguae* cocoons as host material. In contrast to adults of *H. exiguae*, *S. albifrons* adults mate readily in close confinement. Also, the oviposition act is much longer, requiring 3 to 5 minutes as compared with less than a second for *H. exiguae*.

S. albifrons oviposits through the cocoon of *H. exiguae* while the parasite is in the prepupal stage, and it pupates and emerges from the same cocoon. The life cycle from egg to adult takes 23 days at 72° to 76° F. and 30% to 50% RH. Virgin females produce only male progeny, but mated females produce equal numbers of both sexes.

S. albifrons has also been reared as a primary parasite from *Coleophora malivorella* Riley (Beacher 1947). I have also seen specimens of *S. albifrons* reared from the pupae of *Platyptilia antirrhina* Lange (Pterophoridae) by E. I. Schlinger at Riverside, California. He observed oviposition in the pupae of *P. antirrhina* in the absence of any other parasite and its subsequent development and emergence from the same host pupa. Burks (1940) and Muesebeck et al. (1951) listed several other hosts from which *S. albifrons* has been reared as either a primary or a secondary parasite.

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Biology of *Tetranychus "multisetis,"* the Polychaetous Form of *Tetranychus cinnabarinus* (Acarina: Tetranychidae)[1]

DONALD W. DAVIS[2]

ABSTRACT

The mite studied was originally identified as *Tetranychus multisetis* McGregor, which is now regarded as a polychaetous form of *T. cinnabarinus* (Boisd.). No outstanding differences were found between it and either *T. telarius* (L.) or *T. cinnabarinus* in regard to the effect of temperature on each life stage or the sizes of the various instars. Some variations in host range were noted which did not agree with published records on *T. telarius.* Comparative feeding tests were inconclusive. A temperature of about 32° C., with a fairly low humidity, appeared to provide optimum conditions; high humidities delayed hatching of eggs and emergence of active mites from the quiescent stages. Females averaged 67% of the offspring reared from five fertilized females, but the sex ratio varied considerably in the offspring of the various individual parents.

Tetranychus multisetis McGregor was described from specimens taken on beans in southern California, and has since been found in California on cucurbits and other host plants. This form differs from most related mites by the presence of two extra setae on tarsus I of the female. Baker and Pritchard (1953) concluded that this mite does not constitute a valid species in itself but is only a form of *Tetranychus telarius* (Linn.) (= *bimaculatus* Harvey). Boudreaux (1956, 1959) decided, however, that the multisetis form is actually a variant of *T. cinnabarinus* (Boisd.). The mites used in the present work were identified as *T. multisetis* prior to the appearance of the above publications.

The life-history studies made on form multisetis were compared with limited parallel studies and with published reports on *T. telarius, T. cinnabarinus,* and other closely related species. Because of the confusion in nomenclature, the species reported upon by various earlier workers may or may not be the same. Whitcomb (1932) used the name *T. telarius*; Vasser (1938), *T. althaea*; and Cagle (1949), *T. bimaculatus.* All of these names are usually considered synonymous. In addition, the work was carried out in several parts of the world, with mites reared on various host plants.

The habits of form multisetis are essentially like

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[2] The work reported here was done at the University of California, Berkeley, California.

those of *T. telarius*. They are so similar that cross-breeding experiments, using various combinations of related mites, were conducted independently by Davis (1952), Keh (1952), and Boudreaux (1956, 1959). The results showed that copulation took place and both male and female offspring, in a normal ratio, resulted from all the crosses. The offspring from all crosses between *T. cinnabarinus* and form multisetis followed a maternal line of inheritance through a number of generations. Keh (1952) found that a rapid decrease in fertility was usually to be noted in successive generations following inbreeding of *telarius* x *cinnabarinus* offspring.

In the present work, the spider mites were observed and studied on both banana squash fruit and bean leaves, maintained in a modified version of the cages described by Munger (1942) and Huffaker (1948). Most of the studies were made under conditions of controlled temperatures, but without control of the relative humidities. Due to the nature of the cages used for the bean leaves, the relative humidity in them was always high, but this was not the case on the banana squash.

LIFE HISTORY

The eggs were smooth and spherical. Their diameters varied from 0.117 to 0.143 mm., with an average of about 0.133 mm. When first laid they were nearly transparent, but as hatching time approached they became straw-colored, with a reddish tinge. Just before hatching, the red eye-spots could be seen, and the color of the egg was not uniform.

The incubation period varied with both temperature and relative humidity. Under dry conditions at 35° C. an occasional egg hatched in 2 days, but the average was 3½ days. At the same temperature in the leaf cells the average incubation period was 4 days. At 22.2° C. the average was 8 days in the leaf cells and 7 days on the squash, but many eggs required 12 or 13 days to hatch. In a few cases where moisture was allowed to condense, the eggs were killed by only a few hours' exposure to standing moisture. The incubation periods found in the present studies are compared in figure 1 with those obtained by other workers on closely related mite forms. As remarked above, the nomenclatorial confusion makes it uncertain whether these studies actually dealt with the same mite species.

The newly hatched, six-legged larvae were almost colorless, except for the red eye-spots. They were awkward in appearance at first, but soon filled out and resembled small adults to a marked degree. Their size varied with their age; the smallest individual measured was 0.15 mm. long by 0.12 mm. wide, the largest 0.23 by 0.18 mm. The duration of this stage varied from 0.7 to 6 days, depending upon the environmental conditions. As in all succeeding stages, the males tended to develop in a slightly shorter period of time than the females.

Before each molt during their maturation the immature mites became almost completly quiescent for

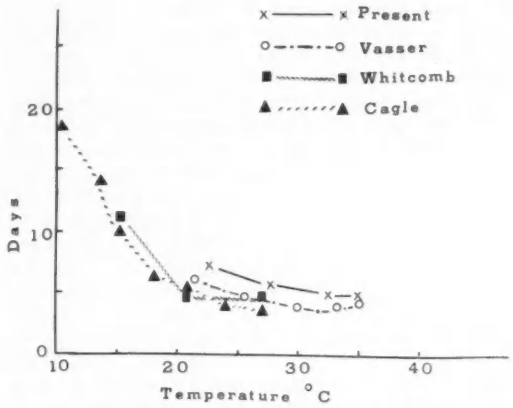

FIG. 1.—Length of the incubation period of the eggs as related to temperature, comparing results of several workers on closely related mite forms.

periods that ranged from ½ to 5 days, depending on the temperature and humidity. The only signs of life at such times were very slight pulsations, which were difficult to observe. Quiescent larvae lay with two pairs of legs pointing forward and one pair to the rear. The quiescent mites were well attached to the host surface by webbing, but whether this was spun by the immatures or whether they took advantage of webbing spun by nearby adults is not known. The average size of the mites was about 0.21 mm. long by 0.16 mm. wide.

Under conditions of extremely high relative humidity, mites of all ages went into an extended period of quiescence. At 26.7° C. such quiescence could be extended up to 10 days beyond the normal period of time without killing the mites. At any time after the normal length of quiescence, these mites molted within a few minutes after being placed in a dry environment. This made it very easy to study the molting process at any time desired.

The active protonymph emerged from the quiescent larva by a transverse splitting of the larval skin which started on the dorsal surface and often resulted in the complete separation of the two halves of the exuvium. In the young forms and in the adult female the anterior part of the body emerged first from the skin, but in the adult male usually the posterior portion was the first to emerge. The nymphal forms had four pairs of legs rather than the three pairs exhibited by the larvae, as the protonymph extracted two pairs from the single posterior pair of the larva. The protonymphs were flesh-colored, and were considerably more active than the larvae. Though there was some indication of sexual differences at this stage, the males tending to be more slender than the females, the sexes could not be distinguished with certainty. The active protonymphs averaged approximately 0.24 mm. long by 0.16 mm. wide, and they remained in this stage from 8 hours to 6 days.

Quiescent protonymphs had two pairs of legs lying forward, and two pairs to the rear. Their average size was about 0.27 mm. long by 0.17 mm. wide, and they remained in the quiescent stage from 15 hours to 8 days. Except for color and size, these individuals resembled the adults quite closely.

The female deutonymph was flesh-colored, with two darker areas along the sides, and was quite active, though less so than the adult. Female deutonymphs remained in this stage from about 8 hours to 16 days, depending on the temperature, and averaged about 0.35 mm. long by 0.20 mm wide. Workers prior to Speyer (1930) failed to recognize the presence of the male deutonymph in the genus *Tetranychus*. Even today many workers follow this old belief, probably because the deutonymph stage in the male is very short and often is not seen. In the present series of studies the active male deutonymphs averaged about 0.27 by 0.17 mm., and spent from about 8 hours to 4 days in this stage—a period considerably shorter than that of the active female deutonymph.

The quiescent deutonymphs retained much the same outlines as the active stages discussed above. The elliptical females averaged about 0.38 by 0.23 mm. in size, the arrow-shaped males about 0.30 by 0.18 mm. The length of time spent in this stage was highly variable, ranging from 16 hours to 29 days for females and from 16 hours to 9 days for males, depending on the temperature and relative humidity. This stage seemed to be even more strongly influenced by environmental factors than any of the other stages.

The adult males, averaging about 0.33 by 0.18 mm., usually emerged from 1 to 3 days before the females. They were very active, crawling over the foliage in search of female deutonymphs that were nearing the end of their quiescent stage. The males apparently were able to recognize such females, and often four or more males waited beside a single one during the last few hours before her emergence.

The adult females were brick-red, with rather heavy setation, and averaged 0.43 mm. long by 0.27 mm. wide. The females were fertilized by the males within a few minutes after emergence; the act of copulation required from 1 to 10 minutes, depending on the temperature. This act, which was described in detail by Ewing (1914) and by McGregor and McDonough (1917), was accomplished by the male crawling under the female and bending his abdomen up over his back. One fertilization was sufficient to last a female for her lifetime, but often copulation occurred more than once. Unfertilized females produced male offspring only, while fertilized females produced both sexes.

Total developmental time is shown in figure 2, where the present results obtained with form multisetis are compared with other workers' studies on related forms of mites. As stated above, the exact identities of the forms studied by others are uncertain because of nomenclatorial confusion. Even among mites reared under the same conditions, the length of the life-cycle was highly variable.

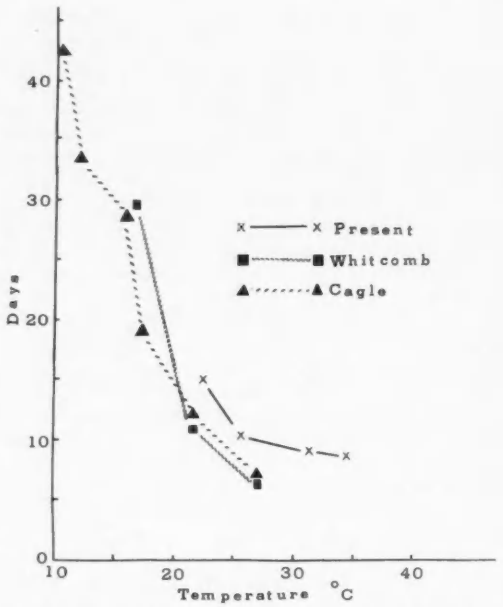

FIG. 2.—Length of total developmental time as related to temperature, comparing results of several workers on closely related mite forms.

Following copulation, there was a preoviposition period that varied from a few hours at high temperatures to about 2 days at 22.2° C. The female laid a few eggs at first, then reached a maximum daily oviposition rate which was maintained for several days. The rate then dropped off quite rapidly, but the female often survived for a number of days longer. Table 1 gives the oviposition data for four females at each of several different temperatures. These individuals were selected from a series of observed females as nearly typical as possible, but were not

Table 1.—Daily egg production at several constant temperatures by the polychaetous form of *Tetranychus cinnabarinus*. X = death of mite.

Day	22.2° C.				26.7° C.				32.0° C.				35.0° C.			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	0	0	0	0	0	0	0	0	0	1	1	2	1	2	0	1
2	0	0	0	0	1	1	0	0	4	3	5	6	6	6	2	7
3	2	0	3	1	1	3	3	3	3	4	10	11	16	12	2	14
4	2	1	2	5	3	4	2	6	7	7	9	8	13	19	6	12
5	2	1	2	4	4	6	3	8	6	7	12	13	8	7	5	7
6	3	3	1	2	5	6	4	5	8	8	7	2	8	14	7	6
7	1	5	1	4	3	7	3	0	5	4	2	5	2	10	5	4
8	2	5	4	0	5	8	0	1	0	6	1	5	7	7	3	5
9	0	1	2	2	0	6	1	1	2	7	0	7	6	6	0	6
10	0	0	1	1	0	3	0	0	1	7	1	6	2	0	0	3
11	0	0	6	2	0	2	0	0	0	3	X	0	2	X	X	1
12	2	0	0	1	0	2	X	X	0	0	–	X	6	–	–	X
13	3	0	0	0	X	0	–	–	0	X	–	–	2	–	–	–
14	2	0	0	1	–	0	–	–	X	–	–	–	–	–	–	–
15	1	X	0	0	–	X	–	–	–	–	–	–	–	–	–	–
16	0	–	0	X	–	–	–	–	–	–	–	–	–	–	–	–
17	0	–	X	–	–	–	–	–	–	–	–	–	–	–	–	–
18	X	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Totals	20	16	16	23	22	48	16	24	38	57	48	65	79	83	30	66

necessarily the extremes. The figure of 19 eggs per day was the highest observed in any of the experiments. At 35° C. a female could lay up to 15 eggs per day for a few days only; at 22.2° the daily oviposition rate varied from zero to five for about 2 weeks, but some females laid eggs during 3 weeks or longer.

The total number of eggs laid was quite variable, but usually was between 30 and 70 per female, with occasional examples up to 120 or more. On an average there were fewer total eggs at 35° than at 32°. Total egg productions in the examples selected for table 1 are not typical in this respect. The temperature optimum for this mite probably was nearer 32° than 35°.

The life span of the adult mites was directly related to the temperature. The females survived about 12 days at 35°, but sometimes lived for a month or more at 22.2°. Males lived a shorter period of time; the maximum length of life observed at 22.2° was 3 weeks, and most males survived for an even shorter period than that.

Mites of this species were highly gregarious. They spun large amounts of webbing which often formed a canopy covering the colony. Most of the spinning was done by adult females, but there was evidence of some spinning done by males, and possibly some also by mites in the older immature stages.

HOST PREFERENCES

A few plants were investigated to determine host preferences, and great differences were found in the number of eggs laid and in the longevity of the adults on different hosts. Waxbean leaves and banana squash fruit were excellent host materials. Strawberry leaves were good, but considerably inferior to wax bean and banana squash. Radish, violet, sugar beet leaves, and citron fruit were all capable of supporting the mites, but were only fair hosts at best. Chrysanthemum, cauliflower, and thimbleberry leaves barely sustained the populations. Hydrangea, azalea, and English ivy leaves did not support the mite populations, and there was no indication that the mites even fed on these plants.

There was also considerable mortality among mites transferred from one host to another, even when both hosts were satisfactory. The mortality when transferred was usually less with adults than with the immature stages.

INFLUENCE OF ENVIRONMENTAL FACTORS

Light had little direct effect on the rate of reproduction or on the longevity of mites; but, when mites were given a choice between light and dark areas of the host plant, they migrated to the dark ones first. As the dark areas became overpopulated, the mites then built up satisfactorily on the light ones.

Except for that of Vasser (1938) and that of Boudreaux (1958), little work has been done on the effect of relative humidities on spider mites. Most workers limited their studies to the effects of temperature. It was apparent, however, that the influences of these two factors can not be separated. Vasser pointed out that there was little effect on *T. althaea* when the relative humidity was varied between 25% and 80%, but at humidities higher than 80% mite development was slowed down and mortality increased; and he also pointed out that the effect of these high humidities was more pronounced under high-temperature conditions than at low temperatures. The present work only touched on the effect of relative humidity, but it is felt that the observations made by Vasser in Russia are valid.

A study of the length of the life cycle and the oviposition rate at different temperatures indicates that 35° C. was above the optimum temperature. The overall reproduction potential of form multisetis seems to be highest somewhere near 32°. This conclusion coincides closely with those of workers who have studied *T. telarius*, except that the optimum temperature may be slightly higher for the polychaetous form. This conclusion, it must be understood, refers to a steady temperature and not to diurnal temperature fluctuations. This mite was able to reproduce at temperatures well in excess of 35° if exposed for limited periods of time only.

Temperature distinctly affected the movements of the individual mites. This is shown in table 2, which summarizes measurements of movements when mites were placed on a new host surface. The rate of travel, distance traveled, and time required to settle down were all greater at the higher temperature.

Table 2.—Comparison of distances and rates of travel of mites at 19° C. and 23° C. on banana squash.

Temp.	No. mites observed	No. mites traveling over 10 cm.	Avg. distance traveled (cm.)	Avg. time for mite to come to rest (min.)	Avg. rate of travel[a] (cm./min.)
19°	18	0	4.78	4.0	2.22
23°	24	16	24.5	6.5	3.62

[a] Excluding time in temporary stops.

SEX RATIO

Various workers on *T. telarius* have given sex ratios ranging from less than 25% males (Worsham 1910) to 45% males (Cagle 1949). In the present study, eggs from five females of the polychaetous form were reared and of these an average of 32.4% produced males. The records of individual females, however, showed variation ranging from 25% to 41.7% males. This indicated that the differences shown by individual parents were too great to permit determination of the exact percentage of males from so few records. Work by Klein (1936) with *T. telarius* showed that there was also a very great seasonal variation in the sex ratio. This, plus individual variation, makes the exact sex ratio difficult to determine.

The sex ratio in established colonies of mites was also checked. Out of 5,264 adults, 79.9% were females. That fewer males are found in a sample taken from a large population than would be expected from the ratios obtained from individual females probably can be explained by the shorter life span of the males.

Age of the female and the time lapse after fertilization had no determinable effect on the sex ratio. Differences due to these factors may exist, but the daily variation in sex ratio of the offspring was too great for any general trend to be detected.

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The Biology, Behavior, and Morphology of *Trioxys (Trioxys) utilis*, an Internal Parasite of the Spotted Alfalfa Aphid, *Therioaphis maculata* (Hymenoptera: Braconidae, Aphidiinae)[1]

EVERT I. SCHLINGER AND JACK C. HALL

Department of Biological Control,
University of California Citrus Experiment Station, Riverside

ABSTRACT

Descriptions and illustrations are given of the adult, the egg, the three larval instars, and the larval head structures, together with illustrations of the male genitalia and of the ovaries and associated structures. Under constant conditions in the laboratory, (1) unmated females produced male progeny only, mated females produced male progeny only, mated females produced both sexes in a 1:1 ratio, and multimated males supplied lesser amounts of sperm in successive matings so that the male-female ratio of progeny went as high as 24:1; (2) though oviposition occurred in aphids of all stages, those of the first three instars were preferred; (3) maximum adult longevity of honey-fed females was 27 days at 50° F.; (4) superparasitism was common, the female apparently being unable to detect a parasitized host, and multiple strikes also were common; (5) reproductive capacity, based on number of eggs per female, ranged between 141 and 179; (6) durations of the various life-cycle stages were: egg 52-53 hours, first-instar larva 9-12 hours, second instar 32-34 hours, third instar 69-71 hours, pupa about 73 hours; (7) first-instar larvae fed almost exclusively on the trophamnion cells and feeding of second-instar larvae was nearly the same, and all contents of the aphid were consumed during the first half of the third instar; (8) with the complete life cycle at 70° F. requiring about 10 days, there are possibly 17 generations per year in the field; (9) a facultative diapause, usually lasting about 120 days, was found during the summer and fall months; (10) aphid defenses against parasitization included the jumping habit of the aphid when disturbed, and production of copious amounts of honeydew which often hindered or trapped the parasite. The jumping habit is partially overcome by the use of the ventral prongs on the parasite's abdomen, which serve to hold the aphid during oviposition. Various factors which may limit the effectiveness of *Trioxys utilis* against its host are discussed, and its present distribution, hosts, and hyperparasites are noted.

The introduction, colonization, and establishment of parasites of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), are briefly summarized by Schlinger and Hall (1959, 1960a) in their papers on the biologies of the aphid's parasites. Suffice it to say that in 1955 three species of parasites (*Aphelinus*

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semiflavus Howard, *Praon palitans* Muesebeck, and *Trioxys utilis* Muesebeck) were shipped from certain Middle Eastern countries to the insectaries of the Department of Biological Control of the University of California for propagation and colonization (van den Bosch 1956, 1957). Biological studies of these parasites were started in 1956-57 and those for *P. palitans* have been completed (Schlinger and Hall 1960a). Since the biology of *T. utilis* differed in certain phases and degrees from that of *P. palitans,* it was decided to report the findings in some detail. Biological similarities of the two species are noted but not discussed at this time.

HISTORY

Little is known about the immature stages and biology of any *Trioxys* species. Apparently, only two articles have been published in which the biology of *Trioxys* species has been mentioned, and each of these has dealt with only a particular phase of development.

Eidmann (1924) was the first to record the habits of adult *Trioxys*. His paper dealt primarily with the oviposition of *Trioxys angelicae Haliday* (as *T. aceris* ? Haliday; see Mackauer 1959). He also described the adult and gave a detailed description of the ovipositor together with its associated parts and their supposed functions. Beirne (1942) discussed and figured the first-instar larva of what he believed was *T. centaureae* Haliday. He mentioned that the second-instar larva was the same as that found in *Praon volucre* Haliday, *Aphidius matricariae* Haliday, and *A. ulmi* Marshall, and concluded that all these species had five larval instars. Mackauer (1959), on the other hand, in his taxonomic revision of the genus stated that there are four larval instars.

MATERIALS AND METHODS

The materials and methods employed in this study were essentially the same as those described by Schlinger and Hall (1960a) for *Praon palitans,* except as follows:

Clearing of second- and third-instar larvae in chloralphenol heated in a water bath was also used with equally good results. After removal from the chloralphenol the larval skin was either mounted without staining directly into Faure's mounting medium, or was treated as described for *Praon palitans.*

First-instar larvae were mounted directly on microscope slides and no clearing or staining was necessary. All drawings were made with the aid of a camera lucida.

DESCRIPTION OF *Trioxys* (*Trioxys*) *utilis* MUESEBECK

Adult (fig. 6)

Muesebeck (1956) based the description of this species on several specimens reared from *Therioaphis maculata* [as *Pterocallidium* sp.] in Italy by R. van den Bosch, and from *T. trifolii* (Monell) in France by H. L. Parker. Living specimens from these localities were sent to the Department of Biological Control of the University of California at Riverside for study. The type locality is Ciampino, Italy, and the type specimen (No. 62983) is in the U. S. National Museum. Muesebeck stated that this species is similar to the European *Trioxys complanatus* Quilis and the North American *T. ameraceris* Smith. However, Mackauer (1959) in his recent revision placed *T. utilis* in the *T. pallidus* group along with *T. betulae* Marshall, *T. pulcher* G. and B., and *T. pallidus* (Haliday) on the basis of the general structure of the abdominal segments, valvulae, and abdominal appendages.[2] A good characteristic of this group is the presence of a single mediolateral, spiracular hump on the petiole.

The chief distinguishing characters of *Trioxys utilis* are the smooth, polished head and the short malar space, which is not more than half as long as the basal width of the mandible. The antennae usually consist of 11 segments in the female and 13 segments in the male. The mesoscutum is smooth and shining, and the prescutellar sulcus is deep and broad; the propodeum is mostly smooth, with only a few weak and indefinite carinae present. The stigma is more than twice as long as wide and the radius is about three times as long as the width of the stigma. The posterior or ventral prongs of the last sternite are nearly straight. The clypeus and mandibles are light brown to piceous; the legs are yellowish brown but are sometimes slightly infuscated; the first tergite and the base of the second are mostly yellow; the remainder of the abdomen is black or dark brown. The legs of the male are usually darker than those of the female.

Male genitalia (fig. 7).—The genitalia are small and consist of the following parts: a long, narrow, apically pointed aedeagus and a pair of long, narrow, diverging basal apodemes; a club-shaped volsellaris which is heavily chitinized and only weakly attached to the indistinct lamina volsellaris by membranous tissue; a chitinized lamina parameralis which partially encloses the base of the aedeagus and bears 10 rather stout setae on the posterior and lateral margins; and a basal ring that is represented by a narrow chitinized band (terminology after Snodgrass 1941).

Ovaries (fig. 8).—Each of the two ovaries consists of a single ovariole held in place by connective tissue. The posterior ends of the ovarioles are swollen and merge with the swollen oviducts. There is no evident line of demarcation between the two. The oviducts are enlarged and hold the fully matured eggs. No fully developed eggs have been found in the ovarioles, and it is thought that final maturation

[2] In a more recent article by Mackauer (Beit. zur Ent. 9: 872, 1959), he stated that *Trioxys utilis* was a synonym of *T. pallidus*; however, we believe the two species are distinct, at least biologically, and may represent sibling species.

of the eggs takes place in the oviducts. The two oviducts unite into a median or common oviduct. The spermatheca with its two accessory glands lies on the median oviduct and enters it by way of an unusually short duct. The alkaline gland, present in *Praon palitans* and certain other Aphidiinae, is lacking in *Trioxys utilis*. Two large poison glands enter the poison duct immediately below the poison reservoir. The poison duct is long, and just before it enters the lumen of the ovipositor near the tip it is enlarged to form what is believed to be an additional pump. The lumen of the ovipositor is of sufficient size to allow for quick, easy passage of the egg.

Because of the minute size of the eggs and the way in which they are packed in the oviducts, a completely accurate count of the eggs was not possible. The number of eggs counted in the oviducts of five virgin females is shown in table 1. The highest number of eggs contained in any one female reproductive system was 179; the lowest number was 141. It can be seen that the total number of eggs per female varies slightly between individuals, but there is little difference in the number of eggs found in the two oviducts of the same female. We were unable to ascertain the exact number of undeveloped eggs in the ovarioles, but they appeared to be few in number.

The number of eggs present in the reproductive system of a newly emerged female of *Trioxys utilis* very closely measured the total reproductive capacity of that female, since it was observed that further maturation of eggs did not take place even though the number of eggs in the oviducts was depleted after a continuous period of oviposition. Females allowed to oviposit freely for 24 hours nearly exhausted the supply of eggs stored in the oviducts. There was no apparent deterioration of the ovarioles after all the eggs had been deposited, as was found in *Praon palitans* (Schlinger and Hall 1960a).

Ovipositor (fig. 8).—The ovipositor together with its associated parts is perhaps the most interesting portion of the female parasite anatomy. At the apex of the abdomen there is a dorsal ovipositor sheath, which encloses the ovipositor, and two ventral "prongs" or processes. The prongs are rigid and slightly curved upward. At the apex of each prong is a small, sharply pointed, articulated finger or digit (fig. 9).

The purpose of the fingers is one of greatest importance to successful oviposition. When the female wasp assumes the ovipositional stance, with the abdomen thrust forward between the legs, the prongs become dorsal and the ovipositor ventral. When a strike is made the ovipositor almost invariably strikes the venter and the prongs strike the dorsum of the aphid. The fingers, which now point down, either penetrate the integument of the aphid or grip it in such a way that the parasite can pull against the fingers and thus fully insert the ovipositor. It is thought that when several eggs are deposited with a single insertion of the ovipositor it is because the wasp is unable to release the grip of the fingers immediately. The deposition of several eggs under these circumstances usually occurs more frequently in older aphids (fourth-instar or adults) which have a much thicker or tougher integument than the younger aphids have, and the fingers are released with greater difficulty. Two or three eggs are usually deposited under these conditions, but as many as ten have been found.

The action of the fingers may also explain multiple strikes or strikes without egg deposition. When the strike is made and the fingers do not obtain a grip on the aphid integument, the parasite then having nothing to pull against is unable to insert the ovipositor. Therefore, if an egg leaves the ovipositor at that time, it is deposited on the outside of the aphid and is lost. The female parasite not obtaining a firm hold on the aphid will then strike repeatedly until the fingers become secure and give the needed support for the insertion of the ovipositor.

Eidmann (1924) stated that the prongs held the aphid and acted as an abutment for the ovipositor. He also stated that suitable host size was somehow determined through the ovipositor.

It is felt that the development of the ventral prongs with their attached, flexible fingers is an evolved, adaptive feature which enables the *Trioxys* species to overcome the saltatorial habits of certain aphid species.

Egg (fig. 5).—The fully matured ovarian egg is translucent milky white. It is roughly elliptical with both ends constricted and bluntly rounded. Both ends are so nearly alike that it is virtually impossible to differentiate the caudal end from the head end. Occasionally the "dorsal" surface is slightly humped, thus giving a curved appearance to the egg. The average length of the fully matured ovarian egg (based on 10 eggs) was 0.073 mm. and the average width was 0.027 mm. The length ranged from 0.066 mm. to 0.081 mm., and the width ranged from 0.021 mm. to 0.03 mm.

The volumetric increase of the eggs after deposition was not calculated because of the extreme difficulty in locating eggs that had been deposited for a period longer than 24 hours. Two other explanations may be offered for not locating the deposited eggs: 1) the chorion of the eggs is very thin and delicate and the eggs are easily broken during the dissection of the aphid, and 2) it has been noted that when the eggs accidentally rise to the surface of the dis-

Table 1.—Egg counts for 5-day-old mated females of *Trioxys utilis* without available hosts.

Specimen	Fully developed eggs in oviduct		Total number of eggs per female
	Right	Left	
1	74	69	143
2	84	65	149
3	84	95	179
4	69	72	141
5	72	78	150

secting fluid, they explode upon contact with the air. There is no doubt in the minds of the authors that there is an increase in the size of the egg due to absorption. The increase is thought to approximate that recorded for *Praon palitans* by Schlinger and Hall (1960a) as 634 times.

Eclosion was not observed, but it is believed to be consummated with the aid of the mandibles of the first-instar larva. This presumption is based on the shape of the mandibles and their mode of action, and is the same as that noted for *Praon palitans* (Schlinger and Hall 1960a). Very soon after eclosion the trophamnion cells were evident in large numbers. At the same time, the adipose tissue of the aphid changed from the characteristic composition to a mass of small globular particles.

Larval Stages (figs. 1-4)

Schlinger and Hall (1960a) published a history of the literature pertaining to the number of larval instars which have been said to occur in the Aphidiinae, and we feel it redundant to repeat them here. The only references to the larval stages of *Trioxys* are those of Beirne (1942) and Mackauer (1959). Beirne mentioned five larval instars, but he figured only the first instar of *T. centaureae*. Mackauer, on the other hand, stated there were four larval instars. He figured only the first-instar larva, and his figure was taken from Beirne.

We are of the opinion that there are three larval instars in *Trioxys* and other Aphidiinae, and that if a fourth instar does occur it is of very short duration and occupies a place between the nonmandibulate second and the mandibulate third instar.

Schlinger and Hall (1960a) discussed their reasons for believing that there are only three instars in the Aphidiinae. They suggested that the occurrence of fourth and fifth instars mentioned by other authors was probably due to the natural growth of the larva, the heavier pigmentation in the facial rods of a later, third-instar larva, and the fact that the forming mandibles of the third-instar larva may be seen through the cleared integument of the late-stage nonmandibulate second instar.

The development of *Trioxys utilis* proceeds from the caudate first-instar larva to the simple hymenopteriform second- and third-instar larvae. The first-instar larva is easily recognized by the presence of distinct mandibles, rows of dorsolateral bristles on the abdominal segments, and a short tail which is covered with spines. The second-instar larva is without mandibles or abdominal bristles, and the tail is considerably reduced in size. The third-instar larva has strong, heavily chitinized mandibles, a loose, rugose integument, and spiracles.

First Instar (fig. 1).—The first-instar larva is easily recognized by the rows of abdominal bristles and the presence of a small but distinctly bristled cauda.

Immediately after hatching, the larva is translucent, but within a few hours it becomes pale milky white in color. It consists of a head and 13 body segments, and is slightly tapered posteriorly. The last thoracic segment and all abdominal segments have a row of small bristles across the dorsum. The rows also extend somewhat along the sides toward the venter. The tail segment is heavily spined and there appears to be no symmetrical pattern in the arrangement of the bristles. The thoracic segments are not greatly differentiated from the abdominal segments. The head is longer than the length of the thoracic segments combined. The venter of the thorax has one or possibly two large lobes, but the dorsum is evenly rounded. The mouth parts consist of a pair of simple mandibles and four fleshy lobes (labium, labrum, and maxillae). The mouth opening is ventral and is between the fleshy lobes.

Internally the oesophagus is clearly visible as a small tube which extends into the first thoracic segment, where it enters the mesenteron. The mesenteron ends blindly in the penultimate abdominal segment. The salivary glands are not easily differentiated in larvae of this stage.

The average length of the first-instar larva is 0.56 mm.; the average width is 0.126 mm.

Second Instar (fig. 2).—The absence of bristles on the abdomen and tail, together with the loss of mandibles, clearly separates this instar from the preceding.

The segmentation of the abdomen is indistinct, and the first thoracic segment is not clearly separated from the head. The terminal abdominal segment is trilobed and asetate. The head is considerably shorter than in the first instar and has a single swelling or lobe on the ventral surface. The mouth parts consist of four large fleshy lobes, and the mouth opening is apical. The integument of the body is quite smooth.

Internally the oesophagus and mesenteron are clearly visible. The mesenteron is distended and ends blindly in the 11th segment. The salivary glands are clearly visible and form a large loop in the head before ending blindly near the oesophagus. The glands terminate posteriorly in the seventh segment.

The average length of the second-instar larva is 0.845 mm.; the average width is 0.168 mm. The average length of the head is 0.165 mm., and the average width is 0.156 mm.

Third Instar (fig. 3).—The body is distinctly segmented, the integument is rugose, and the excess lies in loose folds along the lateral margin. The head is small by comparison with the earlier instars. The mouth opening is dorsal and is flanked by two smooth, sharply pointed, nonopposing mandibles. The orifice of the silk duct is apical. The caudal end of the abdomen is bluntly rounded and consists of a single lobe. Small spiracles are present on all the body segments except the head, the first thoracic, and the terminal abdominal segment. The tracheae are prominent and the salivary gland is clearly visible. Most of the internal mass is obscured from view by the greatly swollen mesenteron. The body is sharply curved, with the bend usually between the first and

second abdominal segments. The length of the mature third-instar larva averaged 1.725 mm.; the width 0.667 mm.

Detailed Structure of the Head (fig. 4).—The structure of the head differs slightly from the typical last-stage larval head of the parasitic Hymenoptera of Vance and Smith (1933), whose terminology is used below. The main points of difference lie in the absence of the metopic suture, the ligular sclerome, and the temporal fossa. The epistoma is not evident nor is the stipital sclerome.

The head consists of a fleshy lobe, distinct mandibles, and strongly chitinized scleromes. The ligular region is snoutlike and is enclosed by the labiostipital ring; within the ligular region are the silk-duct orifice, the labiostipites, and the labial palpus. The maxillary sclerome articulates with the labiostipital sclerome at its anterior end. Posterolaterally the maxillary sclerome articulates with the hypostoma. The pleurostoma forms the articulation for the mandible at the dorsal glenoid fossa and the ventral mandibular condyle. The mandibles are heavily sclerotized, broad, and acuminate; they lie with their apices pointed in, but when in use their tips swing up and the bases then rest along the pleurostoma. Between and slightly anterior to the mandibles is the oral opening. Laterad of the oral opening and enclosed by the hypostomal and maxillary scleromes are the maxillary palpi and the accompanying setae. Posterior to the mandibles is the labrum, which bears two pairs of small setae. Posterior and slightly laterad of the labrum are the antennal buds.

Unfortunately, there are no published descriptions of the head of *Trioxys* species that can be used for comparative purposes. In comparison with other Aphidiinae, *Trioxys utilis* agrees in all essential details and differs only in minor details. The setate labrum and well-separated mandibles are indicative of *Diaeretus* sp. (described as an *Aphidius* sp. by Ullyett 1938, fig. 3). The adult characters of wing venation, weak propodeal carinae, and antennal segmentation also point to a close relationship between species of *Trioxys* and *Diaeretus*.

Cocoon.—The cocoon is formed inside the body of the dead aphid. The silk is laid down in several layers on the inside of the aphid abdomen. As a rule, silk is not laid down within the thorax or head of the aphid. As far as could be ascertained there is only a single envelope enclosing the pupa. The mummified aphid is brown in color and later turns whitish brown to white just prior to emergence of the imago.

The prepupa is similar to the third-instar larva but has a much looser integument, and the fat bodies within the abdomen are clearly visible. The posterior and anterior ends of the prepupa are swollen and bluntly pointed.

The pupa is of the exarate type. It is at first soft and fleshy and pale yellow in color. As development takes place the appendages become visible and the fleshy appearance disappears. Pigmentation is first apparent in the eyes, and the other structures darken gradually. The adult does not emerge from the cocoon until the wings are fully expanded. The last larval molt skin and the meconium are usually found in the middle of the cocoon.

BIOLOGY AND BEHAVIOR OF *Trioxys utilis*

Adult

Emergence of the adult from the cocoon or mummified aphid is effected by using the mandibles and pushing with the head. Emergence holes are almost invariably cut by the mandibles in the posterodorsal portion of the aphid body even though successful emergence can be made from any point except possibly through the venter. Complete liberation requires about 15 to 20 minutes. The adult always emerges from the cocoon with the wings fully developed. Immediately after emergence the parasite runs about, stopping frequently to clean its antennae, wings, and legs. If food is available the parasite usually feeds for a short period. Males are apparently ready to mate immediately upon emergence. The necessity of a premating period in the male was not determined since there was always a certain amount of time spent in searching for a virgin female. The male is apparently unable to detect the female while she is in the cocoon, even just prior to her emergence.

Mating.—The male apparently detects the virgin female by odor and not by sight, which is the case with *Praon palitans* (Schlinger and Hall 1960a). When isolated in a small shell vial with a virgin female, the male immediately becomes aware of her presence even though no physical contact has been made. As soon as sensory contact is made the male begins to run rapidly about, waving the antennae from side to side and raising the wings vertically above the thorax. When physical contact is first made the male vibrates his wings rapidly as they are held above the thorax and runs his antennae over the female. If the male is unsuccessful in his attempt to mate and the female walks away, he follows her diligently even though she may be some distance away. If it is the first union for the male, copulation lasts from 12 to 25 seconds.

Once the female has been mated she apparently loses her characteristic odor and is no longer attractive to other males unless they accidentally make physical contact with her.

Females were found to mate only once during their lifetime. When males were placed with recently mated females, they were always discouraged from mating. The female either ran away or bent her abdomen under so that copulation could not take place. A rather common act of discouragement on the part of the female is one of striking at the male with the ovipositor.

Multiple matings are evidently normal for males. One male was observed to mate with nine different virgin females, and no doubt would have mated with more if they had been available. Although it was not determined how many times a male would mate, it was noted that each successive union took a shorter

period of time. For example, the male noted above remained united with the first virgin female for 12 seconds, whereas the ninth or final mating lasted only 3 seconds. The series of matings took place from 5 minutes apart to 1 hour and 30 minutes apart. The later series of matings did not occupy any longer periods of time between them than did the first three or four matings.

Progeny and Sex Ratio.—Virgin females were found to produce only male progeny. Females that were the first or second matings of a male usually produced progeny in the sex ratio of 1:1. The male evidently depletes his sperm supply rapidly with each successive mating, since a female that was ninth in a series of matings by a single male produced progeny in the sex ratio of 49 males to 2 females. In the laboratory stock of parasites, a sex ratio of 1:1 was usually easy to maintain if the parasites were allowed to mate freely for at least 24 hours before their collection and use.

Reproductive Capacity.—Maturation of the eggs takes place before and after emergence. Approximately 75 percent of the total complement of the eggs are fully developed when the female emerges from the cocoon. Thus no apparent preoviposition period is required. Females that are confined without hosts and food for 5 days retain their full complement of eggs, thus showing no indication of ovisorption. As noted in table 1, the total number of eggs per female ranged from 141 to 179 (average, 152). When provided with an adequate number of suitable hosts the females are capable of depositing their full complement of eggs. The average reproductive capacity for the species is thus about 152-fold.

Ovipositional Habits.—Many peculiarities in the ovipositional habits of *Trioxys utilis* were observed. Among them may be mentioned multiple strikes (a strike is here considered to be that act of the parasite whereby the ovipositor is thrust toward the aphid, with or without making contact), supernumeraries, stinging without egg deposition, and the deposition of several eggs with a single insertion of the ovipositor.

The female parasite was able to detect the presence of the aphid by sensory contact, odor, and sight. The presence of a colony of aphids is probably detected by some odor given off by the honeydew or the aphids themselves. Female wasps were observed to walk with their heads down and palpi in contact with the leaf surface. The palpi are thought to be used in locating the honeydew on which the parasites readily feed. The aphids themselves are not touched with the palpi. When an aphid is located the parasite does not touch the aphid with the antennae except on rare occasions. One female was observed to play her antennae over the aphids encountered without touching them, but she did not sting these aphids. Later, when this female was ready to oviposit, she did so without touching the aphids, including those aphids she had rejected previously.

When contact with an aphid is made, the female parasite quickly strikes at it with the ovipositor in the manner of other aphidiine wasps. Only the ovipositor and ventral prongs touch the aphid during parasite oviposition. If the ovipositor is not inserted, the parasite backs away and moves around the aphid to another position; or occasionally she strikes again in the same area. Even though the ovipositor has evidently been inserted, the female often continues to strike again and again. A possible explanation for this phenomenon is given above in the discussion of the reproductive organs. The parasite does not orient herself to sting any particular part of the aphid, but oviposits in the host from whatever angle she happens to approach. Most of the stings occur in the abdomen, as this portion makes up the bulk of the aphid.

The female parasite usually stings four to six aphids in rapid succession if hosts are available. The female then locates another aphid, approaches it and raises her wings to a vertical position and rapidly vibrates them. Females have been observed to stand in this position from 3 to 5 minutes before striking. When the female is ready to sting again she usually repeats a similar group of stings before resting again. After each successive series of stings the rest period becomes a little longer and the number of stings in each successive group diminishes, until near the time of the wasp's death there is a rest period of nearly 2 hours, and as few as four to six stings are noted. As the parasite ages her reaction time in oviposition slows down. A young female will leave the ovipositor inserted for 1 to 2 seconds, while the older female may leave the ovipositor inserted for as long as 20 seconds. The rest periods noted above are thought to be due to the time lapse necessary to bring the eggs from the oviducts to the lumen of the ovipositor. The frequency with which a female oviposits is dependent to a large degree upon the density of the host population and the age of the parasite. A freshly emerged female, when placed with a large population of aphids, oviposited with a frequency ranging from 30 seconds to 4 minutes. The aphids were struck from 1 to 10 times. The female parasite often returned to aphids previously stung and struck at them several more times.

Because of the habit of the female of *Trioxys utilis* in not touching the aphids with the antennae before ovipositing, multiple stings are commonplace. This habit was especially pronounced in a low, restricted population of aphids. The female parasite is apparently unable to distinguish a previously parasitized aphid whether the aphid was stung by herself previously, by another female of *T. utilis*, or by another species of parasite. One female parasite within a period of 10 minutes stung a single third-instar aphid 14 times—once in the thorax, four times in the abdomen, and nine times in the head. This aphid died within 24 hours. Eidmann (1924) found that in *T. angelicae* (as *T. aceris*) the tips of the ovipositor sheath, or cerci (see fig. 10), possessed sense organs which served to detect a parasitized aphid (fig. 10). Eidmann found no case of super-

parasitism. We feel that since he apparently made no dissections to see if there were supernumerary eggs or larvae present, superparasitism may occur in *T. angelicae* as well as in other species of *Trioxys*.

Supernumerary eggs and larvae were very often found when dissecting aphids. In one aphid which was stung only once in the abdomen, nine first-instar larvae and one second-instar larva were found. Only the second-instar larva was alive. The number of eggs or larvae usually encountered in the dissections was two.

Under restricted laboratory conditions the common occurrence of multiple stings and multiple egg deposition with a single insertion of the ovipositor causes the efficiency of *Trioxys utilis* to be greatly reduced, so much, in fact, that a single female may, on occasion, realize only 50 percent of her reproductive potential. This unfavorable condition is not thought to occur in more favorable field conditions.

Adult Longevity.—To determine the approximate longevity of *Trioxys utilis* at different constant-temperature and humidity conditions, an experiment was set up using a small shell vial with either five virgin females or five virgin males (with honey added for food) for each of several temperature points from 28° to 100° F. The relative humidities used for this experiment were those available in the various temperature units and ranging from 34 percent at 28° to 86 percent at 50°, 60 percent at 60° and 70°, 20 percent at 80° and 90°, and 30 percent at 100°. The humidities seemed satisfactory except in the higher temperature ranges (80° to 100° F.). Higher humidities at these temperatures probably would have yielded greater longevity.

A temperature of 50° F. and relative humidity of 86 percent gave the greatest longevity for both males and females (25 and 27 days, respectively). Tests at 60° and 70° F. showed a greater longevity than those at 28°, 80°, 90°, or 100° F. The increases in longevity at 28° to 50° F., and the decreases at 50° to 60° F. were of about the same magnitude.

A test similar to that described above, but without honey or food of any kind, showed the importance of food for this parasite's longevity. The maximum longevity of a female at 70° F. was only 1 day. Comparable reductions in longevity were found at other tested temperatures.

Since very little reproduction of *Trioxys utilis* was obtained at 50° F., and since the longevity at 70° F. was only about half of that at 50° F., it follows that a temperature of about 60° F. and a relative humidity of about 60 percent are the best conditions to maintain for rearing this parasite.

Host Size Preference.—Observations in the field and in the insectary indicate that females of *Trioxys utilis* oviposit in all stages of the spotted alfalfa aphid. The stages of aphids preferred are apparently those of the first three instars, as these were most commonly found containing eggs or young parasite larvae. Eidmann (1924) also found that aphids in the first three stages were the ones preferred by *T. angelicae*. In the field the adult aphids usually were not stung unless the aphid population density was low and the parasite density high. The reluctance of *Trioxys* females to parasitize adult aphids, especially alates, certainly reduced the natural spread of the parasite in California. According to Eidmann (1924), host size discrimination or selection by females of *T. angelicae* resulted through the ovipositor and only aphids of a certain body size could be successfully stung. As stated above, this was not found to be the case in *T. utilis*.

Superparasitism.—This phenomenon is quite common with *Trioxys utilis*, since females of this species are apparently unable to distinguish previously parasitized aphids. This was in contrast to that found in *Praon palitans* to a certain extent (Schlinger and Hall 1960a). Occasionally, several eggs were deposited with a single insertion of the ovipositor. In one instance when an aphid was stung only once in the abdomen and then removed from the parasite and dissected 76 hours later, nine first-instar larvae and one second-instar larva were found. Only the second-instar larva was alive. Two or three live first-instar larvae have been found in a single aphid, but only a single parasite was ever found to develop to maturity in one aphid.

Elimination of Supernumeraries.—The elimination of supernumerary larvae (internecine action) is apparently brought about in two ways: 1) by mechanical means—that is, by the direct feeding of one larva upon another; and 2) by the destruction of rivals through chemical means.

Evidence of the mechanical destruction or elimination of rival larvae is based upon two observations made in which the actual feeding of one larva on another was seen. The body contents of the victims were observed to pass through the oesophagus and enter the mesenteron of the victor. The body of the rival larva does not collapse but remains in its original state because at this time only enough of the body contents are consumed to kill the larva. If the observations had not been made on the actual feeding of one larva on another, it would have been very difficult to prove that this took place as no apparent marks were left as a result of the feeding. Since the mandibles of the first-instar larva are not used in feeding, no detectable injurious marks are left on the victim.

The majority of dead larvae seemed to have been killed by some chemical means. This was apparent when a dead larva and a live larva were found in widely separated parts of the aphid, as, for example, one in the thorax and the other in the abdomen. That the larvae are killed very soon after eclosion is evidenced by the trophamnion cells which still partly surround the dead larva. It is interesting to note that the trophamnion cells of the dead larva do not develop and have nearly the same appearance as the dead larva.

It is thought that the first larva to hatch excretes an inhibitory substance which acts to kill or stop the

development of any other larvae as soon as they emerge from the egg. The existence of a chemical secretion of some sort is evidenced also by the rapid change in the ovarioles (and embryos) of the host a few hours after the parasite larva emerges from its egg. It was not determined, however, whether or not the larvae that were being fed upon were alive or dead at the time of attack by the rival larva. Internecine action of the parasitic larvae is quite obvious, whether mechanical or chemical means are employed, since not more than a single individual ever emerged from one host.

Egg

The general features of the egg are discussed above in the descriptive portion of this paper. Eggs found in aphids did not appear to be attached to any definite part of the aphid anatomy. Eggs deposited in the abdomen of the host were found floating free in the lumen of the body; those deposited in the thoracic region were usually embedded in the muscle tissue, where they remained until eclosion. It was not determined whether or not the egg traveled through the aphid body by the circulation of the haemolymph, but eggs deposited in the lumen of the thorax were, upon dissection, often found in the abdomen. Eclosion took place within 52 or 53 hours at 70° F. No difference in the time of eclosion was found in relation to the region of egg deposition.

Larvae

Observations on the habits of larvae of *Trioxys utilis* have shown that they do not differ in any great respect from the habits of larvae of *Praon palitans*, described in detail by Schlinger and Hall (1960a).

The first-instar larva molts 60 to 62 hours after egg deposition, or 8 to 10 hours after eclosion. The second-instar larva molts approximately 94 hours after egg deposition, and the length of this stadium is 32 to 34 hours. The third-instar larva kills the aphid approximately 120 hours after egg deposition, and within 5 hours after the death of the aphid the larva cuts a small slit in the venter of the aphid's abdomen, attaches a few minute strands of silk to the leaf surface, and begins spinning the cocoon. The larva never extrudes more than the length of its head through the slit in the aphid body.

Cocoon Spinning

Because the cocoon of the parasite is actually made within the body of the aphid, complete construction of the cocoon was not observed. The first threads of silk are attached to the inside of the aphid skin and are then stretched and fastened to the substrate, pulling that section of the aphid skin close to the substrate. This process is repeated around the opening in the aphid body until it is completely pulled down and sealed against the substrate. After the edges of the slit are fastened down, the larva begins laying strands of silk across the venter until it becomes nearly opaque. The opening into the thorax of the aphid is silked over and sealed. It was not determined just how many layers of silk are then laid down over the inner surface of the aphid's abdomen, as the cocoon often became so opaque that the larva could not be seen.

As far as could be ascertained, the cocoon is completed within 12 to 15 hours after the aphid's skin is split.

Pupal Period

The parasite larvae becomes quiescent after spinning the cocoon and remains so even after the meconium is passed. The meconium consists of 12 to 15 small oval black pellets and is passed approximately 30 to 35 hours after the start of the cocoon, or about 152 hours after egg deposition. The molt into the pupa takes place from 2 to 13 hours after the meconium is deposited. The larval exuvia is in the form of a flattened scale and is found near the apex of the pupal abdomen.

The third-instar stadium was 69 to 71 hours in duration. The mature larva molted 163 to 165 hours after egg deposition. Structural and color changes in the pupae of *Trioxys utilis* did not differ from those described for *Praon palitans*. The total length of time spent in the pupal stage was approximately 73 hours or 3 days.

Adult Emergence

The adult parasite usually emerges from the cocoon with little difficulty. A small circular hole is cut with the mandibles in the posterior portion of the mummified aphid, usually just above the cauda. As in the case of *Praon palitans*, mortality during emergence appeared to be higher than at any other time. Humidity apparently plays a large part in the successful emergence of the adult. If the humidity is too low, the wings never fully develop and the adult is unable to free itself completely from the cocoon. The adult emerges from 236 to 264 hours or about 10½ days after egg deposition, or about 112 hours after the cocoon is started. It is of interest to note that the developmental period of *Trioxys utilis* averaged 1½ days shorter than that for *P. palitans* on the same host species (Schlinger and Hall 1960a).

RESUME OF LIFE HISTORY AND GENERATIONS PER YEAR

Under the laboratory conditions (temperature 70° ± 5° F., and R. H. 50 to 60 percent) the minimum time for a complete generation from egg to adult was 236 hours or nearly 10 days. The maximum time under the same conditions was 264 hours or 11 days. The life history may be broken down into the following stages and periods: egg stage, 52 to 53 hours; first instar, 9 to 12 hours; second instar, 32 to 34 hours; third instar, 69 to 71 hours; and pupal period, approximately 73 hours.

Under these laboratory conditions, allowing 11 days for each generation, about 32 generations a year

could be produced. Under variable field conditions the life history may be faster or slower than one generation in each 11 days. Dispause is in effect for about 120 days a year in southern California. At an average of 14 days per generation with time for mating and for parasite search for suitable hosts, 17 generations per year could occur in the field.

A table of dissections similar to that given for *Praon palitans* by Schlinger and Hall (1960a) was made for *Trioxys utilis*. Because of the similarity of the two tables, it seems unnecessary to give more than a summary of the table for *T. utilis*. Sixty-two dissections were made, and the following remarks are based upon these dissections. The number of ovipositional strikes made at any one aphid ranged from one to seven; the average number of strikes for the 62 observations was 1.6. Tabulation showed that only seven ovipositions were made in the head and only three in the thorax; the remaining 52 were made in the abdomen. As stated above, these data do not show that the abdomen was the preferred area for oviposition, only that the abdomen, which makes up the bulk of the aphid, is the area most likely to be struck by the ovipositor. The number of young aphids deposited by aphids that were parasitized ranged from 0 to 13, the average being 1.5. Young aphids were deposited only by those aphids that were stung while in the fourth instar or adult stage. Two exceptions occurred with aphids stung in the third instar, but a possible explanation for this could be that the two aphids were in a late third instar when stung and molted to the fourth instar soon afterwards.

REACTIONS OF HOST TO PARASITE AND PARASITIZATION

The host aphids reacted in much the same way to adult females of *Trioxys utilis* as did those described by Schlinger and Hall (1960a) for *Praon palitans*.

The aphids apparently have two means of defense against the parasite— 1) the production of copious quantities of honeydew which often entangles adult parasites, and 2) the ability to jump when disturbed. The latter defense is more typical of adult aphids.

Very little reproduction occurs when an aphid is parasitized. Young aphids were deposited by those aphids stung in the late fourth-instar and adult stages. In a series of experiments the number of young produced by parasitized aphids ranged from 0 to 13 with an average of 1.5 per adult aphid. Aphids stung when in the first or second instar never produce young, and adult aphids produce only a fraction of their potential.

After the parasite egg hatches, the aphid is reluctant to move about as normal unparasitized aphids do, and when the parasite larva reaches the third instar, the aphid moves only with considerable prodding.

DIAPAUSE

Field and laboratory investigations have shown that *Trioxys utilis* has a facultative summer diapause (Schlinger and Hall 1960b). No detailed diapause studies have been carried out by the authors, but certain features pertinent to this study have been noted.

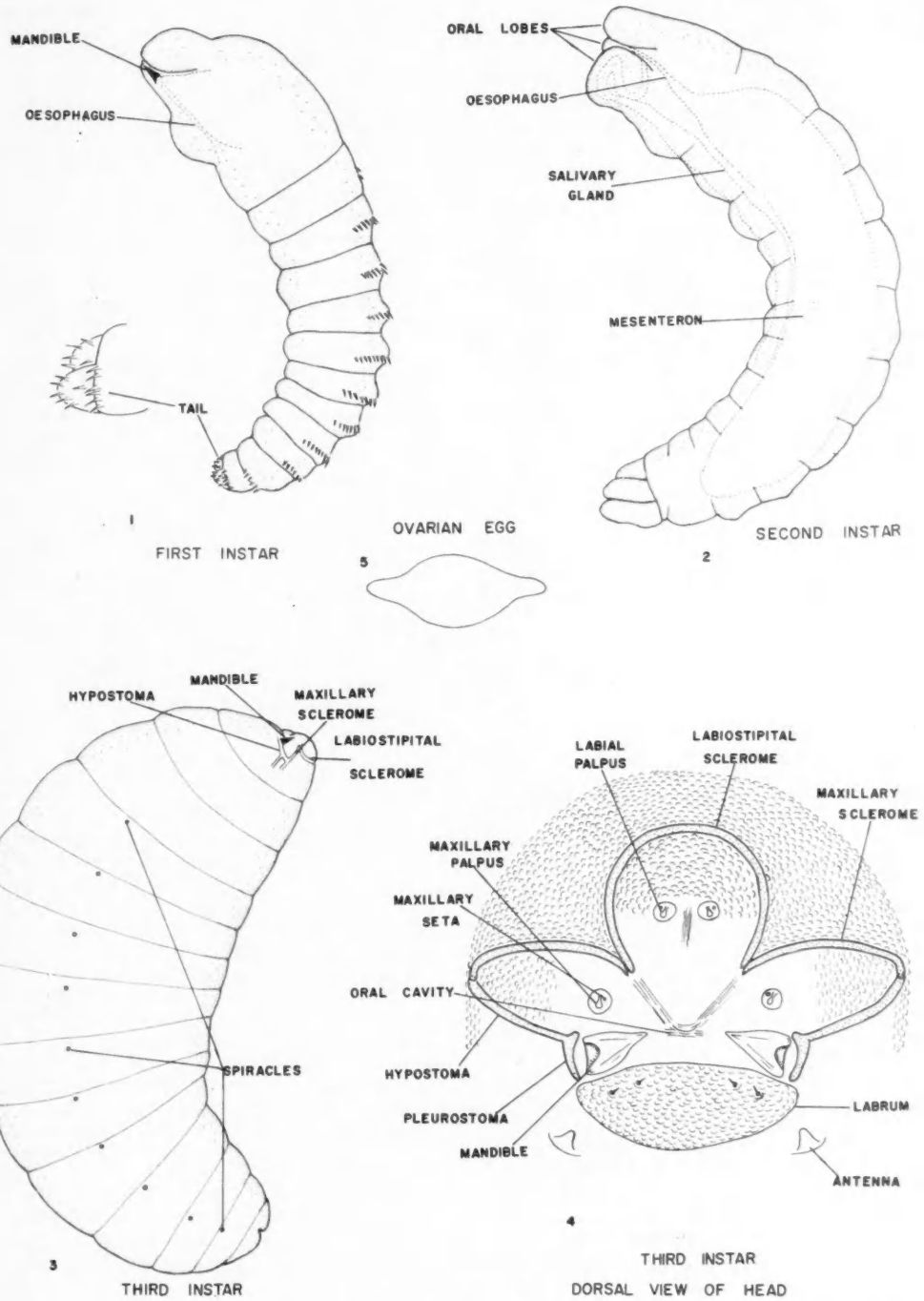
The diapause state of the parasite is spent as a mature larva inside the cocoon, which at first glance does not appear to differ greatly from the normal nondiapause cocoon. Closer examination, however, shows that the color remains dark brown instead of turning white as in nondiapause cocoons, the cocoon is much tougher, and the slit in the venter of the aphid body is sealed over so that the larva inside is not visible. In the areas in southern California in which the diapause state was investigated, it was found that diapause usually lasts from June or July of one year through February or March of the following year. Because of climatic conditions, diapause may last only from July to October in some areas.

The individuals of any one diapause population apparently emerge about the same time even though these individuals entered diapause over a longer period of time, often of several weeks. The response of all the individuals to the stimuli that break diapause takes place regardless of when they entered diapause. In the Imperial Valley of California it is not uncommon to find small areas or pockets where there are few or no diapausing individuals. Diapause in the Imperial Valley is apparently synchronized with the reduction or cessation of aphid reproduction during the hot, dry summer, as borne out by dissections of adult aphids. In the more northern areas (such as the San Joaquin and Antelope Valleys of California), where extremes of high temperatures are not experienced, adults of *Trioxys utilis* may be found throughout the year. In the intervening areas between the north and the south, a few individuals may be found to enter diapause but the population as a whole appears to remain active most of the year.

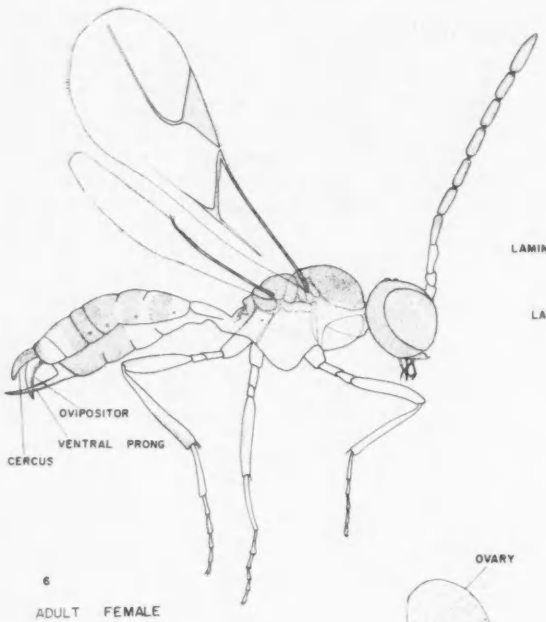
Emergence of the parasites from diapause in the spring is usually well synchronized with the beginning of the spring aphid population buildup, and heavy parasitization takes place on the first and second generations of low host density. The parasite remains effective at various rates until diapause occurs in June and July. On the other hand, if diapause is not broken by October, the parasites are almost totally ineffective against the fall build-up of the aphid populations. *Trioxys utilis* is perhaps in better synchronization with its host in those areas which do not exhibit such extremes of heat and dryness. However, up to 1960 *T. utilis* was the only one of the three imported parasites of the spotted alfalfa aphid which was able to survive the hot, dry summers in the Imperial Valley since the time of the original establishment of these parasites. In 1960 both *Praon palitans* and *Aphelinus semiflavus* were able to reproduce in the Imperial Valley under an improved, experimental cultural program (Schlinger and Dietrick 1960).

FACTORS LIMITING THE EFFECTIVENESS OF *Trioxys utilis*

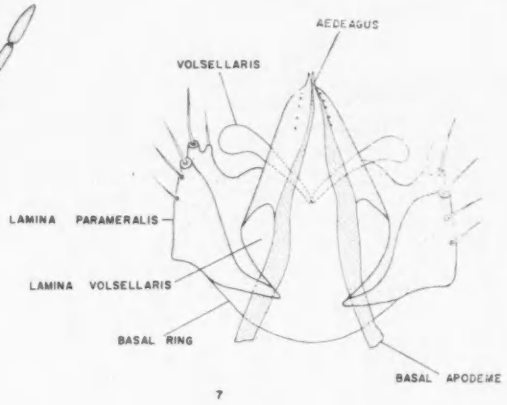
Nearly every factor limiting the effectiveness of



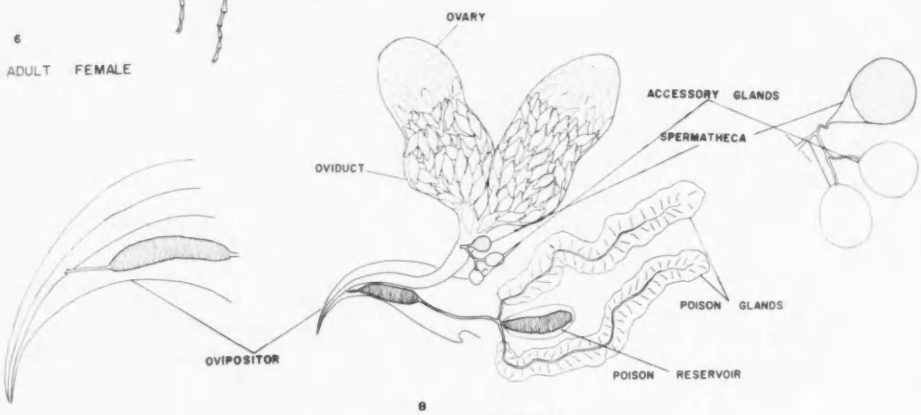
FIGS. 1-5.—Egg, larval stages, and larval head structure of *Trioxys utilis* Muesebeck (not drawn to scale).



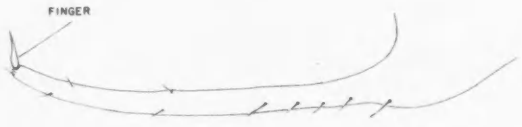
6
ADULT FEMALE



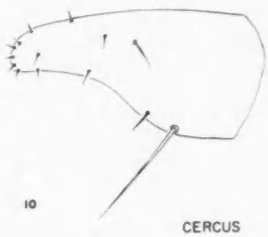
7
MALE GENITALIA



8
FEMALE
REPRODUCTIVE SYSTEM



9
VENTRAL PRONG



10
CERCUS

FIGS. 6-10.—Structural details of adult *Trioxys utilis* (not drawn to scale).

Praon palitans, as discussed by Schlinger and Hall (1960a), is applicable to *Trioxys utilis*. Among the limiting factors that may be mentioned here are: Competition from other spotted alfalfa aphid parasites (*Praon palitans* and *Aphelinus semiflavus*), and from Coccinellidae, other aphid predators, and fungus diseases; destruction by hyperparasites; period of nonoccurrence due to diapause; and the sporadic effects of climate, dispersal, and farming practices.

Diapause and climate are sometimes effective in limiting the efficiency of *Trioxys utilis* in those areas which have extremely hot, dry summers. Although the parasite is able to survive under such conditions, synchronization with the host is occasionally poor and not advantageous to the biological control of the host.

Trioxys utilis, unlike *Praon palitans*, is not transported to any great extent as larvae within alate aphids. Since *T. utilis* prefers to sting aphids of earlier instars and the life cycle is much shorter than in *P. palitans*, the hosts are commonly killed before aphid wings are formed. During the summer and fall of 1956 in the Antelope Valley of California, *T. utilis*, principally by its own powers of dispersion, spread over an area of about 10 square miles as compared to an area of 700 square miles for a similar period of time of dispersal of *P. palitans* (van den Bosch et al. 1957). As reported by van den Bosch et al. (1959a) and by Schlinger and Hall (1959), the natural spread of *T. utilis* was comparatively slow. However, the development of a mechanical collector (van den Bosch et al. 1959b) facilitated its spread and establishment throughout California in a short period of time.

DISTRIBUTION, HOSTS, AND HYPERPARASITES OF *Trioxys utilis*

Distribution.—At present this parasite is established in all the alfalfa-growing areas of California. It is also established in parts of Arizona, Nevada, New Mexico, Utah, and Mexicali, Mexico. As reported by van den Bosch (1957), its distribution in the Old World includes western Europe, parts of the Mediterranean area, and many Middle East countries.

Hosts and Hyperparasites.—In the United States only the same species of the genus *Therioaphis* as reported for *Praon palitans* (Schlinger and Hall 1960a) are known to be hosts of *Trioxys utilis*. The same hyperparasites which attack *P. palitans* also attack *T. utilis*, namely, *Pachyneuron siphonophorae* (Ashm.), *Asaphes californicus* Gir., *Aphidencyrtus aphidivorus* (Mayr), and three or four species of *Charips*.

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The Dimensions of Endemism[1]

RICHARD L. DOUTT

University of California, Berkeley

ABSTRACT

Endemic populations have dimensions other than of space and time, for there are also measurable characteristics at the genetic and trophic levels. The latter feature is of considerable importance to the procedures followed in biological control, particularly in the search for effective natural enemies of an insect host and the subsequent attempts to establish these parasites in a new area. These hypotheses are discussed in relation to field studies made on an endemic insect, *Rhopalomyia californica* Felt, and its parasites in association with an endemic food plant, *Baccharis pilularis* DeCandolle.

It is generally recognized that biological control of agricultural pests is concerned for the most part with exotics, i.e., with introduced invertebrates or adventive weeds which constitute the majority of our agricultural pests. Furthermore, for the suppression of these alien pests we deliberately introduce still other exotic organisms; the parasitoids, predators, or weed-feeding insects. It might therefore be said with considerable accuracy that the biological control aspect of the entomological profession is primarily a commerce in exotics.

Dictionaries indicate that exotic is the antonym of endemic. Accordingly, it may seem anomalous to suggest that workers in biological control who deal essentially with exotics should actually give serious attention to endemics and the subject of endemism. However, as will be pointed out herein, there is no paradox or contradiction in the notion that in order to understand and intelligently employ exotics in biological control projects a person should study the anatomy of endemism. Accordingly this paper reports the results of research on an endemic host and its parasites and reviews the literature on this general subject.

Biological control is applied ecology, and as most entomologists know it is based on the use of certain organisms that function as mortality agents in naturally occurring populations. Our exotic pests are usually somewhere endemic, and in biological control we attempt to reproduce, to duplicate in a sense, the mortality aspect of the pest's native biotic environment. In theory, and in practice as well, the procedure in biological control is to return to the indigenous host population to find the most effective and presumably the best adapted natural enemies. Accordingly, when an organism has become a pest following its introduction into a new area, great importance is immediately attached to the parasitic fauna associated with the native population of the species. Substantial expenditures of money and technical efforts are made on the basic premise that with the indigenous population will be found the most effective natural enemies.

This belief seems to be fundamentally correct, for each project initiated in biological control against an exotic immigrant pest has been undertaken with the supposition that the native home of the pest is the most profitable one to explore. The soundness of this assumption is reflected in the results that have been achieved during the past 70 years in many parts of the world. Another way of emphasizing this point is to consider biological control projects merely as a series of replicated experiments to test this single idea. Viewed in this light, the results conclusively show that this hypothesis has been proved.

It is obvious therefore that the characteristics of an endemic population and the interrelations of its natural enemies form the very core of biological control work. Accordingly it is singularly surprising that entomologists, particularly those in biological control work, have not examined endemism per se, but have instead derived their concepts from collateral and oblique observations rather than from direct investigational approaches to the subject. Consequently, the study of the characteristics of a purely autochthonous population has not been a usual practice in our entomological research, and so this novelty, coupled with the desire to examine certain concepts regarding the relative dimensions of endemic populations of hosts and their attendant parasite populations, has furnished incentive for the present investigation.

There is relatively little literature on endemism itself, and those entomologists who do write about it are either taxonomists, or are biogeographers, and, as such, are entirely dependent upon systematics.

A taxonomist who has mastered his subject is interested in the distribution of the species in his group. He uses these data in conjunction with other evidence to build a concept of the phylogenetic relationships and the general history of the organisms with which he works. The true systematist is not satisfied until he can visualize his special group in time and space.

A biogeographer is a compiler of data who integrates the distributional studies made by many individual taxonomists. He gives particular emphasis to areas in the world where there may be a high incidence of endemism, and these summarizations, generally of adaptive radiation, are of great interest and significance to all students of evolution and speciation.

In view of the fact that endemism is of common interest to people in many fields of natural history it is perhaps surprising that biologists do not all agree upon the meaning of the term endemic. The Webster unabridged dictionary says that endemic

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in biology means "confined to, or indigenous in, a certain area or region." In medicine it means "peculiar to a district or particular locality, or class of persons; as an endemic disease. An endemic disease is one which is constantly present to a greater or less degree in any place, as distinguished from an epidemic disease, which prevails widely at some one time, or periodically, and from a sporadic disease, of which a few instances occur now and then."

Babcock (1947) in his monographic study of the plant genus *Crepis* writes, "The term endemic, in its usual biological connotation, is applied to a plant or animal which is limited in its distribution to a single country, region, or area. Of equal or perhaps greater importance is the extent of the organism's actual range of distribution within the endemic area."

Darlington (1957) defines endemic as "confined to; occuring nowhere except in the place in question." Darlington goes on to say, "This word has other meanings and should be used cautiously. I would not use it at all except that a very useful noun is formed from it, *endemism*, the existence of endemic forms. Synonyms: exclusive, peculiar, precinctive."

Babcock (1947) stressed the importance of "the extent of the organism's actual range of distribution within the endemic area." Like most botanists, and unlike most entomologists, he was able to measure with great precision the limits of the distribution of his species. Because of this he classified his endemics into "narrow" endemics and "wider" endemics. His arbitrary division was based on a linear dimension of 50 kilometers (31 miles). Anything restricted to a smaller area was a "narrow" endemic. Mason (1946a, 1946b) also uses the term narrow endemics for such restricted species, but apparently he does not fix them within a given measurement of area. The plant geneticist Stebbins (1942) calls such restricted distribution an example of "extreme" endemism.

Zimmerman's (1948) outstanding analysis of the distribution of the Hawaiian insects should be read carefully by an entomologist interested in endemism. Zimmerman speaks of *native* insects in Hawaii and recognizes two categories. He classifies them as *indigenous* and *endemic*. His category of endemic species includes those entirely restricted to the Hawaiian Islands, even at times to a single island, whereas he considers the indigenous insects as those that live naturally in Hawaii as well as elsewhere. From his point of view of the Hawaiian fauna this may be a very valuable distinction, but from the general standpoint of endemism his two types of native insects are merely expressions of the extent of the distribution of endemic species. In other words, his endemic category is comparable to the "narrow" endemics of the botanists, and his indigenous category is comparable to the "wider" endemics.

This is not intended nor should it be construed as a criticism of the very admirable paper of Zimmerman. However, the word indigenous might better be thought of as being a synonym of the wonderfully descriptive word, autochthonous, which refers to a species being native in the sense of having originated, i.e. evolved, in the place in question. An autochthon is one supposed to have risen or sprung from the ground or from the soil he inhabits; one of the original inhabitants or aborigines; a truly native or indigenous plant or animal.

At this point a discussion of the center of origin of a species would seem to merit an extensive treatment, but this is being deliberately avoided, because many of the criteria that have been used to determine such centers will not stand alone. In fact, Kinsey (1936) thought we were using these criteria to find what never existed. It may not be of importance, really, to find the center of origin, but it is of utmost importance in biological control work to locate the endemic population of a pest species. This may sound illogical, but actually a species may be restricted in its distribution to a particular area and yet it may not necessarily have originated there. Plant geographers, for example, often make a dichotomous split of endemic species into (1) those that are young endemics and (2) those that are relict species. These relict species were formerly widespread and now exist only in restricted areas. The *Sequoia* species in California are famous examples of such relicts.

At this point another dimension to endemism appears. Previously it was shown that one of the characteristics of an endemic is its distribution as far as area is concerned, but now it is evident that there is also a distribution in time. Therefore there is a time dimension to endemism.

Willis (1922) proposed the so-called "age and area" hypothesis. His idea was that the distribution of an organism was an expression of age, and the longer it had existed the greater its range. Willis believed that endemic and rare species are beginners and have not had time to spread. This hypothesis has been subjected to scathing criticism and is generally discredited.

Hultén (1937) proposed an interesting theory called equiformal progression. His idea is that a species starts at one point and spreads at a constant rate in all directions until it reaches conditions which check its further spread.

Fernald (1924) was one of the critics of the age and area theory and said that most rare species were once common, but that their great age and the vicissitudes to which they have been subjected have made them conservatives and unable to spread.

Stebbins (1942) concedes that while conservatism rather than aggressiveness may be a characteristic of rare plants it is not necessarily the result of senescence or the age of a species. Stebbins indicates that the same species may be rare and conservative in one part of its range and common and aggressive in another.

Stebbins is strongly inclined toward a genetic concept of rare species based on the view that most widespread and common plant species consist of a large number of genetically different biotypes, many of

which differ widely in their ecological preferences. This is really the ecotype concept, which conceives of these widespread species as consisting of several clusters of similar biotypes, each cluster, or ecotype, differing from other ecotypes in its ecological tolerances. Therefore if a species with many ecotypes and biotypes is widespread and common it follows that a rare species in this genetic concept will be one poor in biotypes, and with its few biotypes so specialized that they can grow and compete with other species in only a limited area.

Under Stebbins' view aggressiveness, or the ability of a species to colonize new areas, and to crowd out other species, is the result of the possession of a great store of genetic variability either evident or concealed. This consists of genetic heterozygosity, of biotypes preadapted to new conditions which the species might encounter, or of a rapid mutation rate by which the new biotypes may be produced. A species is conservative on the other hand, if it contains few biotypes, most of which are homozygous or nearly so, and has a low mutation rate.

Stebbins stresses this idea of genetic homogeneity in narrowly endemic species. He believes that they consist of relatively few biotypes which are themselves homozygous, and he contrasts them with the common and widespread species which have great genetic heterogeneity, and possess a great store of genetic variability. According to Stebbins there are two types of these homogenic rare species and the two types (depleted and insular) reflect the two possible conditions of the past history of a rare species. The depleted species was once more common, widespread and richer in biotypes than now, so that its present rarity is due to depletion of the store of genetic variability. Some authors say that such a species is depauperated with regard to its biotype contents, but Stebbins thinks depleted is a better term. The insular species, on the other hand, was never common but diverged from a small group of individuals of a widespread ancestral species, following the establishment of these individuals upon a small insular area.

Kinsey (1937) stresses the existence of these insular species among the Cynipidae. He points out, as does Stebbins, that insular species can exist on continents as well as islands. He considers 76% of the gall wasps on the North American continent to be insular species. Kinsey noted the great uniformity among the insular forms and said that his studies verified the theory of Sewall Wright, that near homogeneity should be reached more quickly in a small, freely interbreeding population than in a large, partially subdivided group.

The endemic Hawaiian insects, now known to number more than 3,700 species, are believed to have arisen from ancestral stocks of about 200 species. Zimmerman (1948) says that "One of the most significant points to keep in mind here is the lifting out of a population of a single fertilized individual from which may spring a new and isolated population series and eventually an array of forms. The impregnated female carries only the genotype of herself and her mate, not the genetic makeup of an inbreeding species population. She does not represent an average of her population. The implications and potentialities of such action are great." Zimmerman further points out that we are dealing initially with small populations. "It is these small populations which restrict gene flow and encourage random divergence where nonadaptive characters can flourish, and these populations exist in an environment favorable to the survival of changed or changing forms."

The established procedure in biological control is to process in quarantine the shipments of imported material collected by an entomologist in the foreign field. The parasites which emerge from this imported host material are used to start the initial cultures of the beneficial species. It is not uncommon to begin such a stock culture with only one or two females, for that may be all that emerge from a shipment. The progeny from this tiny beginning are bred for several generations and are the ancestors of the millions of individuals that are eventually colonized in the field. If the above quoted authors are correct, then the implications of using the progeny from such a small stock are indeed great. We may be establishing a parasite that only partially resembles the parental species in the native home. The insects which we are colonizing may thus not be truly representative of the species. This, however, is a two-edged sword and has potentialities either way. We may be establishing an entity that is superior to the wild stock, or we may be colonizing a genetic strain that is not as efficient as the parental species. Through this reasoning, and from the findings of the cytologists who have studied the wild populations of *Drosophila* and *Chilocorus* in the United States, it is evident that there is a genetic dimension to endemic populations.

Thus there are clearly several dimensions to endemism, namely one of space, one of time, and one of gene pattern. There is still another dimension and one which is of especial significance to biological control, for it is seen at the trophic levels. Workers in biological control have noticed that the parasitic fauna of any given host seems to vary considerably at different times and in different places. This does not necessarily mean that such an observation reflects a proven fact because such an impression could be gained if one were basing his view on inadequate and incomplete samples, or if collecting were done at different seasons, or even at different times of the day. Assuming, however, that this great variability in time and space of the host and various elements of its parasitic fauna does in fact exist, one can note then in analysis that such observations have been made in the course of projects involving pest species with comparatively widespread distributions and pests which attack a substantial number of different host plants in many ecological situations. Under these conditions a biologist would logically expect to find diversity in the associated parasitic fauna. Since

this diversity has been observed among the parasites of widespread and polyphagous pests one may query whether or not this is a fundamental characteristic of host-parasite populations. Several questions spring to mind immediately: Is there a differential distribution of the parasitic fauna at the endemic locus of a species? Is there a differential distribution of the parasitic fauna associated with those species that are not widespread nor polyphagous? Do the relative dimensions of an endemic host population and its complex of parasitic species tend to be equivalent widely variable?

Apparently it has been taken for granted in biological control circles that there is such a diversity, and it also appears that these concepts have actually bridged the gap from hypotheses to guiding principles without having beeen subjected to critical examination. It is not only good science, but in an applied field it is also good economics to appraise the ideas which have a strong influence on the planning and conduct of field work. This is obviously a question of more than mere academic interest, because it has some direct bearing on the procedures followed in foreign exploration for natural enemies and in the subsequent colonization and evaluation phases of any specific project in biological control. If this differential distribution among the parasites of an endemic host should really prove to be an inherent characteristic of such a host-parasite relationship, then biological control workers would always be compelled to take recognition of it in formulating policies and procedures. For example, in the foreign exploration for natural enemies it is important that all the members of a parasitic complex are obtained. Therefore, assuming that such diversity is a fact, it will be necessary to search completely throughout the natural range of the host and through its entire seasonal cycle before one can positively say that all the natural enemies have been found. By the same token, in the subsequent colonization of parasites in the domestic field it will be necessary to test each imported species under all types and combinations of field conditions before one can say with assurance that all possibilities of establishment have been exhausted. Finally, in evaluating the action of natural enemies in regulating the population density of any host it will be necessary to take this differential distribution of parasites in time and space into account if our ultimate conclusions are to have validity.

There is then another dimension of endemism reflected in the food chain. It is a concept of endemism upon endemism, i.e., an endemic host plant fed upon by an endemic insect which in turn has its endemic parasites. This assemblage of interdependent organisms might be thought of as a pyramid of endemics. There is very little in the literature on this aspect or dimension of endemism, but there is one splendid example from Hawaii, that wonderful area of endemics where 99% of the native insects are narrow endemics. Zimmerman (1948) gives several indices of endemicity, and as one of the criteria points out that most endemic Hawaiian insects are confined to native forest plants. He notes varying degrees of host plant specificity and he gives some astonishing examples among phytophagous forms. This is not unique to Hawaii, however, for Kinsey (1937) found in the Cynipidae that only 0.003% occurred on anything but their normal hosts.

Zimmerman (1948) says "The small leaf-mining *Philodoria* moths include at least six which are attached to the widespread endemic urticaceous tree *Pipturus albidus*. Most of these species are not known to overlap in distribution. Thus, one is known from Kauai; 3 from Oahu, two of which are restricted to small areas in different mountain ranges and one which is widespread; and two from Hawaii, but these occupy widely separated localities in different mountain ranges." These remarks are indications of the first step in the food chain, namely the phytophagous insect often does not extend throughout even the limited range of its endemic host plant.

It is evident that competition, predation, and parasitism are generally of low intensity on islands like Hawaii, and these influences have favored explosive types of speciation. The pressure is low because of the great difficulties involved in overseas dispersal and colonization. It has been only of very rare occurrence that phytophagous insects have managed to cross the extensive sea barriers and become established, and rarer still have been the successful invasions by predators and parasites. They are one step up the food chain, and the arrival of predators and parasites without the previous establishment of suitable hosts obviously would lead to the early death of any successful immigrants. As a practical matter in biological control there are times when considerable difficulty is encountered in establishing the purposely introduced forms even under the best of conditions.

According to Zimmerman (1948), even predaceous insects are frequently confined to particular host plants. He gives an example of a nabid, *Nabis kahavalu* (Kirkaldy) which is confined to *Sophora*. Since there are few data on the comparative dimensions of this step in the food chain in relation to the endemic host plant and its endemic herbivores, it was considered particularly worthwhile to investigate an endemic community representing these three steps in the food chain. California, like Hawaii, is a favorable area in which to study endemics. Jepson in 1925 listed 1,416 endemic plants in California (approximately 40% of the flora) and in 1946 Mason pointed out that additional plant surveys and better taxonomic techniques had increased the known endemics to a considerably higher figure. In spite of this ample endemic plant base and the presence of numerous endemic insects, it was necessary nevertheless to make the selection of a particular study community with some care. It was decided finally to focus attention on the gall midge, *Rhopalomyia californica* Felt, which attacks the native Coyote Brush, *Baccharis pilularis* DeCandolle.

This particular host insect species was chosen for several reasons: (1) It is restricted to one plant species, *B. pilularis*. (2) The midge is multivoltine, and thus continues to breed uninterruptedly throughout the year without complications caused by diapause. (3) The insect induces the formation of conspicious terminal galls (figure 1) on the *Baccharis* plants, and consequently this enables field sampling to be done without great difficulty and provides a good population index. (4) The midge has a substantial number of parasitic species associated with it (5) The host plant, *B. pilularis,* has a range which is sufficiently extensive to cover a variety of climatic conditions and yet is so geographically restricted as to permit surveys throughout its entire area of distribution. (6) Finally, the host plant, the host insect, and its parasites are all amenable to laboratory culture, and this facilitates detailed studies on the biological relationships of this community.

Many of the details of the study that was made on *Rhopalomyia californica* are not within the proper scope of this paper. Accordingly only those aspects which have some bearing on the subject of endemism are discussed here.

THE ENDEMIC PLANT HOST

The Coyote Brush, *Baccharis pilularis* DeCandolle, is in the family compositae and is one of nine species of the genus *Baccharis* that occur in California. These are mostly shrubby, or at least suffrutescent, perennials all of which are dioecious, insect pollinated, and abundant seed producers that rapidly take possession of disturbed areas.

Baccharis pilularis consists of two distinct subspecies. *B. pilularis typica* is a prostrate shrub, forming mats about 6 inches high and spreading laterally 6 to 15 feet. The main stems are flexible, rarely over ½ inch in diameter. The branchlets are mostly turned to one side, reaching upward from the prostrate branches. The subspecies *typica* ranges along a narrow coastal strip of California from Russian River in Sonoma County southward to Point Sur in Monterey County. It usually occurs on exposed and windswept sand dunes and bluffs, but this is not to imply that its characteristic growth form and structure result merely as a response to the local coastal environment, for it seems to be a true maritime subspecies as it retains its identity when artificially grown away from the coast. Along most of its range, *typica* overlaps with the other subspecies, *consanguinea,* and intermediates are found where the two have naturally hybridized.

The subspecies *consanguinea* is an erect or rounded shrub varying in height from 3 to 12 feet. The main stems are stiffly erect, often 1 to 3 inches in diameter with the branchlets rather evenly disposed around the branches which are not flexible as in *typica* but tend to be brittle. This subspecies ranges along the coastal strip from Coos County in Oregon to San Diego County in southern California. Offshore it is found on the Channel Islands of San Miguel, Santa Rosa, Santa Cruz, Anacapa, and Santa Catalina. Along much of its range it can be found a few miles inland. The greatest inland ex.ension occurs east of Sacramento where it occurs sporadically in the Sierra foothills from Nevada County south to Tuolumne County.

According to Tilden (1951a) the ecological position of the species is not easily fixed. Certainly it is evident that the species occurs in a transect across California where very steep climatic gradients occur within a few miles. Thus whereas *typica* forms part of the wind-swept flora of the cliffs, dunes, and terraces near the sea where summer fogs are heavy and prevalent, its relative *consanguinea* extends inland where the summers are hot and dry and the winters are not tempered by the immediate maritime influence.

The plant growth is dependent upon available moisture and *Baccharis* will continue to put out new growth throughout the year if water is available. Normally its growth pattern begins in the fall with the onset of rains and will continue until the dry summer season when the soil moisture is depleted. *Baccharis* is an evergreen and there are varying rates of leaf fall throughout the year. The plants near the coast flower from August to December, but inland the blooming period is shorter. The seeds are equipped with pappus and are widely dispersed by wind currents. The seeds will germinate in as short a time as two days. Growth of the seedlings is fairly rapid under favorable conditions. The life span of the plants in the field is on the order of 10 years (Tilden, 1951a).

THE ENDEMIC INSECT HOST

The gall making midge, *Rhopalomyia californica* Felt, is the endemic species which forms the center of this investigation. It develops in conspicious, many-chambered galls on the plant terminals (figure 1). The females oviposit large clutches of vermillion colored eggs in the terminal shoots of the plants (Tilden, 1951b). This oviposition occurs irrespective of mating, but the infertile eggs do not hatch. The fertile eggs hatch in 3 to 4 days, and the tiny larvae

FIG. 1.—Terminal galls induced by larvae of *Rhopalomyia californica* Felt on *Baccharis pilularis* DeCandolle.

work their way into the buds and leaf axils. If conditions are favorable the typical gall will have been formed in 15 days and the larvae will begin pupating 30 days after oviposition. This is, however, about the maximum rate of development, which is actually so variable that it is difficult to determine the norm.

The physiological condition of the plant determines the rapidity of gall formation, and this in turn seems to govern the rate of development of the larval stages of *Rhopalomyia*. Even in the areas where gall midges abound it is common to find many plants which are uninfested. This seems to be a result of the physiological condition of the plant at the time being adverse to gall formation rather than to genetic resistance, for tests on hundreds of seedlings in the laboratory have indicated that all plants are susceptible to attack. Although midge eggs are deposited in the terminal bud areas, the galls do not develop unless the plant is in a favorable cycle of growth. This factor seems to have been an important one in determining the density of the galls in many areas. Generally speaking, near the coast and where the maritime influences extend inward along rivers and creek drainages through the coastal hills, and where the host plants do not suffer long periods of growth cessation, the galls are common. Beyond the first coastal ridges, where the coastal fog rapidly dissipates and the temperatures and humidities undergo more extreme variations, the plants do not find such favorable periods of growth, and the density of the galls is noticeably less than near the coast and in the strictly maritime belt.

Observations also indicate that the most favorable locations for gall formation are not on the windswept sea cliffs and fronts directly exposed to the sea, but in slightly less exposed areas which may be a few yards to several miles inland, but which are still within the moderating influence of the maritime air. This is probably an expression of two factors, (1) a more favorable growth pattern of the plant coupled with (2) conditions which permit the midges to oviposit more frequently and for longer periods of time without interruptions.

The geographical range of the gall midge is of considerable interest in relation to the subject of endemism. It was found to occur on the host plant from the most northern collections in Del Norte County south to Santa Barbara County. None, however, were found from Santa Barbara southward, although the host plant is not uncommon there along the coast and extends inland for a few miles up the major riparian areas. On the other hand, the geographical range of the midge extends eastward across the Sacramento Valley and into the Sierra Foothill counties. The absence of the midge in the southern extension of the host plant range is surprising, and no satisfactory explanation for this is at hand.

PARASITE COMPLEX ASSOCIATED WITH *Rhopalomyia californica*

Twelve species of parasitic wasps were found to attack *R. californica* (table 1). The superfamily Proctotrupoidea is represented by three species of the single genus *Platygaster*, and these three species are also the only internal parasites in the entire complex.

The superfamily Chalcidoidea is represented by species in five different families. All of these chalcidoids are external parasites of the midge larvae or pupae. The predominance of external parasites is explained by the protected habit of the host. The host larva is enclosed in a hard-walled, cylindrical cell in the gall. The larva is never exposed or unprotected after the formation of the gall, and in this situation there is no advantage to the parasite to adopt a mode of internal existence. By contrast, the three internal parasites attack the host during the egg stage when it is exposed and subsequently develop internally in the larva as it enters the bud and develops in the gall.

Table 1.—List of parasitic Hymenoptera reared from *Rhopalomyia californica* Felt.

Superfamily Proctotrupoidea
- Family Platygasteridae
  - *Platygaster californica* (Ashmead)
  - *Platygaster lampronota* Fouts
  - *Platygaster* sp.

Superfamily Chalcidoidea
- Family Torymidae
  - *Torymus baccharicidis* (Huber)
  - *Torymus pilularidis* (Huber)
  - *Torymus koebelei* (Huber)
  - *Torymus* sp.
- Family Pteromalidae
  - *Amblymerus* sp.
  - *Meraporus* sp.
- Family Eulophidae
  - *Tetrastichus* sp.
- Family Eupelmidae
  - *Eupelmus inyoensis* Girault
  - *Eupelmus* sp.

Laboratory tests showed that each of the 12 species of parasites developed as primary parasites of the gall midge. It is possible, however, that some could develop either as primaries or secondaries, but none exhibited a predilection for the hyperparasitic habit, and it is remarkable that no secondary parasites were found during the testing of this complex.

Evaluation of the parasite complex.—In conducting this study the galls were collected in the field and isolated singly into cotton-stoppered vials. The emerging hosts and parasites were recorded by number, species, and sex. Many of the galls produced neither hosts nor parasites. Apparently, the inhabitants of these galls were not sufficiently developed to mature and emerge. Of 1700 field-collected galls, 37% produced nothing and were blanks. One hundred laboratory-produced galls were collected as a comparison and 41% of these were also blanks, indicating that the sampling method had the same influence on both naturally occurring and artificially reared galls.

For the purpose of a general analysis 1000 field-

collected galls that did produce gallicoles were tabulated (table 2). Of these, 956 produced parasites and only 44 produced hosts without parasites. This would indicate that an extremely high percentage of the galls (95.6%) are found by one or more species of parasites. Although there seems to be no single hallmark of endemism, it is generally agreed in biological control work that an endemic host population will have a large and effective parasite complex. These figures give substantial support to this belief. It should be pointed out that this degree of parasitism is the amount which was present at the moment of sampling, and any further parasitism of the same galls was precluded by the removal of the galls. It is quite possible that had the galls been permitted to remain in the field many of the 44 parasite-free galls would have been attacked.

Table 2.—Emergents from 1000 field-collected galls.

	Galls producing	Number
(a)	No parasites, only hosts	44
(b)	No hosts, only parasites	843
(c)	Both hosts and parasites	113
(d)	Only *Torymus koebelei* (Huber)	117
(e)	Only *Torymus bacharicidis* (Huber)	49
(f)	Only *Torymus* sp.	64
(g)	Only *Torymus pilularidis* (Huber)	17
(h)	Only *Amblymerus* sp.	44
(i)	Only *Meraporus* sp.	24
(j)	Only *Tetrastichus* sp.	27
(k)	Only *Platygaster* spp.	78
(l)	Only *Eupelmus inyoensis* Girault	24
(m)	Only *Eupelmus* sp.	2

An analysis was made of the proportion of galls accounted for by each species singly as well as the proportion of the galls which had a combination of two or more species of parasites. Since each species can occur in a gall to the exclusion of the others it is presumed that each species has habits which enable it to work in situations not encroached upon by other parasitic species. Each has some niche in which other members of the complex do not compete with it. This would seem to indicate that none of the species are ecological equivalents and consequently all are able to survive even though competitively inferior in many respects to other species associated with the same host. Even the rarely encountered *Eupelmus sp.* is able to find hosts which escape the more dominant species in the same locality.

This is evidence strongly supporting the policy followed in biological control of introducing all of the primary parasites of a given host rather than a single dominant species. Smith (1929) contended that the entire complex of natural enemies will be able to account for a greater number of hosts than a single dominant species because the generally inferior species are always able to find certain hosts in niches not frequented by other parasites.

There are several possible approaches to the determination of the order of importance among the parasite species as they occur in the endemic population of the host. One approach is to consider the relative proportion of the galls which produce only a given species. By this criterion it was found that *Torymus koebelei* is the most dominant species. Apparently it is able to find its hosts more readily than the other species. Another basis is to measure the frequency of occurrence of the species in the collections, irrespective of whether the species occurs alone or in combination with others. By this method of evaluation *T. koebelei* was again found to head the list. A third method is to give weight to the numerical abundance of the species, but this is a method subject to much criticism, involves the matter of sex ratios, and is the least desirable criterion. On this basis *T. koebelei* fell to third place.

When these three methods are combined in a single appraisal of the ordinate ranking of the members of the complex it was found that *Torymus koebelei* is the dominant species, *Platygaster californica* is second, followed by *T. bacharicidis*. This is probably very close to the case generally, particularly when the distribution of the species is considered. Both *T. koebelei* and *P. californica* range throughout the distribution of the midge, whereas *T. bacharicidis* tends to be much more active and abundant near the coast than it is inland or at the extreme fringes of the host range.

An attempt was made to determine whether particular associations between the parasitic species existed. In order to do this each species was considered in relation to every other member of the complex and the total number of times the various species were found to be associated in the same galls was recorded. From these combinations the data showed that certain members of the complex occurred together more frequently than others, and this in turn reflected the ecological preferences of the species. For example, the *Amblymerus* and *T. bacharicidis* associations are common along the coast, and although they do occur inland and in the more extreme situations, associations with *T. koebelei* are much more common there.

DISCUSSION

The gall midge, *Rhopalomyia californica*, although specific to the endemic *Baccharis pilularis*, does not occur throughout the range of this host plant. The plant's range extends 100 to 200 miles beyond the southern limit of the gall fly.

The growth pattern and physiological condition of the plant is an important factor in limiting the number of galls.

The parasite complex of *Rhopalomyia californica* consists of 12 species of parasitic wasps, representing 2 superfamilies, 5 families, and 6 genera. At least three of the species are undescribed.

The distribution of parasites is not the same throughout the range of the host. Certain parasites are very limited in distribution while others with wider ranges nevertheless exhibit distinct ecological preferences and have areas where they are more

abundant and important than elsewhere. Still other species are found generally and commonly throughout the entire range of the host. Examples of species limited to a small portion of the host range are *Eupelmus sp.*, found only on the coast, and *Platygaster* sp., found only in the Sierra foothills. Both *Amblymerus* and *Torymus bacchariciclis* are more active on the coast than elsewhere in the range. *Torymus koebelei* and *Platygaster californica* are commonly encountered throughout the host's range.

The parasite complex contained no obligatory hyperparasites, and all of the species were found to occupy some niche not encroached upon by other members of the complex. This is submitted as evidence strongly supporting the contention that in biological control it is better to introduce all available primary parasites of a given host than to introduce one or two dominant species.

The parasitism of the endemic galls is so high that most field-collected galls produce no host insects. Only 4.4% of the galls were without parasites, and it is probable that these would have been subjected to parasitism if they had been allowed to remain in the field.

The suggestion is made that the study of the characteristics of an endemic population is important to the clarification of concepts which influence the policies governing biological control. It is suggested that there are several dimensions to endemism, namely spatial, temporal, genetic, and trophic.

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The European Corn Borer, *Pyrausta nubilalis*, and Its Principal Host Plant. IX. Biological Activity of Chemical Analogs of Corn Resistance Factor A (6-methoxybenzoxazolinone).[1]

STANLEY D. BECK AND EDWARD E. SMISSMAN[2]

University of Wisconsin, Madison

ABSTRACT

Benzoxazolinone and 6-methoxybenzoxazolinone, found in the tissues of several plant species of the family Gramineae, play an important role in host-plant resistance to insects and fungi. Biological activity is measurable as inhibition of growth of the insect or pathogen. A study was made of the growth inhibitory properties of over 50 analogs of benzoxazole and related phenolics, using the European corn borer, *Pyrausta nubilalis* (Hbn.), and *Penicillium chrysogenum* as test organisms. Fungal growth inhibition was found to be dependent on two structural characteristics of the molecule: (1) the presence of a lipid-solubilizing group on the benzenoid nucleus, and (2) the presence of a nitro or amino group adjacent to a phenolic hydroxyl. Antifungal actvity was not enhanced by an oxazole ring. Growth inhibitory activity against the European corn borer was closely associated with the presence of an oxazole or thiazole grouping, and phenolic compounds were of generally low activity.

Benzoxazolinone and its 6-methoxy analog have been recently reported as naturally occurring chemicals in four species of the plant family Gramineae. Koyama et al. (1955) isolated 6-methoxybenzoxazolinone from roots of a number of species of the genus *Coix*. They assigned the substance the name *coixol*.

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[2] Present address: Department of Pharmaceutical Chemistry, University of Kansas, Lawrence.

Of 40 genera of Gramineae examined, the chemical was detected only in *Coix*, and only in the roots (Koyama 1956). Virtanen and Hietala (1955) reported the isolation of benzoxazolinone from the foliage of seedling rye, identifying it as an antifungal factor implicated in plant resistance to *Fusarium nivale* infection. 6-Methoxybenzoxazolinone was found to occur in the foliage of wheat and corn seedlings by Virtanen et al. (1956). The same substance was independently isolated and identified as being involved in the resistance of corn plants to infestation by the European corn borer, *Pyrausta nubilalis* (Hbn.) (Loomis et al. 1957, Smissman et. al. 1957, Beck and Stauffer 1957). Because other, unidentified, chemicals also appeared to play a role in corn plant resistance to the corn borer, 6-methoxybenzoxazolinone has been referred to as Resistance Factor A (RFA), (Beck 1957, Beck and Stauffer 1957).

In addition to *Fusarium nivale*, benzoxazolinones have been shown to inhibit the growth of *Fusarium moniliforme* (Sheld.) (Whitney and Mortimer 1959) and a number of other *Fusarium* species (Beck, unpubl.). Benzoxazolinones also inhibit growth of the fungi *Penicillium chrysogenum* (Beck and Stauffer 1957), *Diplodia zeae* (Schw.) (Loomis et al. 1957, Whitney and Mortimer 1959), *Gibberella zeae* (Schw.) (Whitney and Mortimer 1959), *Rhizopus stolonifera* (Beck and Stauffer 1957), *Sclerotina trifolium* (Virtanen and Hietala 1955), and *Pyrenochaeta terrestris* (Hansen) (Whitney and Mortimer 1959). In addition to the European corn borer, benzoxazolinones have been shown to inhibit the growth of the following insects: *Blattella germanica* (L.), *Prodenia eridana* (Cram.) (Beck and Stauffer 1957) and *Galleria mellonella* (L.) (Beck, unpubl.). The bacterium *Staphylococcus aureus* was also inhibited by 6-methoxybenzoxazolinone (Beck and Stauffer 1957), but no other bacterial species were tested. It is apparent that this class of compounds may have importance in host-plant resistance to a number of insects and microorganisms.

According to Virtanen and coworkers (Virtanen and Wahlroos 1958, Virtanen and Hietala 1959, Wahlroos and Virtanen 1959) benzoxazolinone and 6-methoxybenzoxazolinone do not occur as such in rye, wheat, or corn plants, but are formed enzymatically from unidentified glycosidal precursors when the host tissue is crushed or damaged. The evidence for such transformation is far from definite, however, and whether or not benzoxazolinones occur in undamaged plant tissue must be considered an unsettled question. From the standpoint of plant resistance to either insects or fungi, the problem is of interest, as it bears on the question of whether such resistance factors are pre-existing in plant tissue or are formed as a result of tissue damage inflicted by the invading organism. In either case, these substances appear to play an important role in host-plant resistance.

The present report concerns the growth-inhibiting properties of a series of benzoxazolinone analogs, in an effort to establish some of the structural relationships involved in their biological activity. To the writers' knowledge, the only previous tests of benzoxazole analogs for biological activity against either insects or fungi were the studies of Siegler et al. (1939), and Swingle et al. (1944). Both studies involved inclusion of a small number of benzoxazole derivatives among compounds screened for insecticidal potency. Most were found to be of very low order of toxicity, although it was noted that 2-phenylbenzoxazole was quite toxic to first-instar larvae of the southern armyworm, *Prodenia eridania* (Cram), and to some species of termites.

METHODS AND MATERIALS

The substances tested in this study may be conveniently divided into four related series. Their names, position numbering systems, and structural formulae are shown in figure 1. The authors are

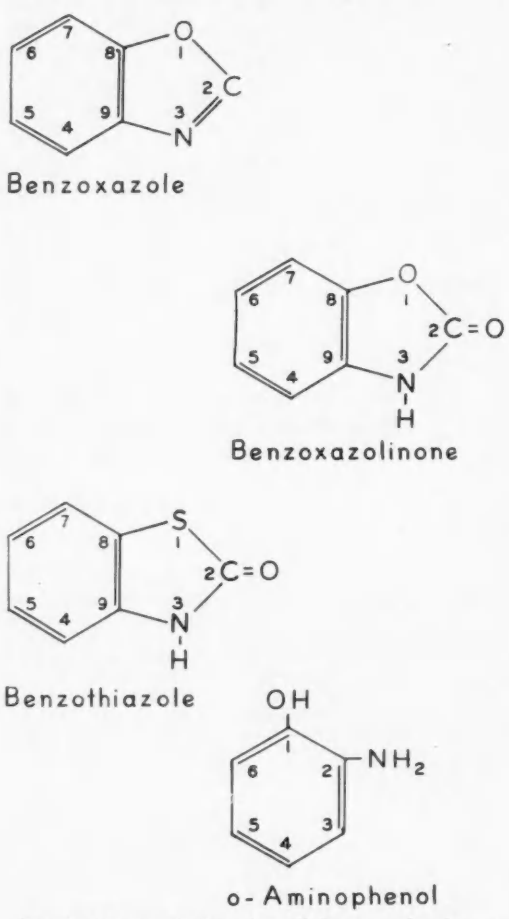

FIG. 1.—Structural formulae of benzoxazole, benzoxazolinone, benzothiazole, and o-aminophenol.

pleased to acknowledge their indebtedness to Abbott Laboratories, McNeil Laboratories, and Merck, Sharpe, and Dohme Company for providing a number of the compounds employed.

SYNTHETIC METHODS

A number of the compounds were synthesized by methods other than well-established procedures. The synthetic methods devised are presented below.

6-Methylbenzoxazolinone.—*m*-Cresol was nitrated by the procedure of Clemence (1934). The 2-nitro-5-methylphenol obtained from the reaction was reduced catalytically and fused with urea in the manner previously reported (Smissman et al. 1957) for the synthesis of 6-methoxybenzoxazolinone. The resulting compound was recrystallized from water, m.p. 142°-143° C. The elemental analyses calculated for $C_8H_7NO_2$ was: C, 64.66; H, 4.69; that found was: C, 64.41, 64.49; H, 4.72, 4.79.

5-Chlorobenzoxazolinone. — 2-Nitro-4-chlorophenol (Eastman, tech.) was reduced and fused with urea as in the above procedure. The resulting product was crystallized from water to give white crystals melting as 189°-190° C. Elemental analysis: Calculated for $C_7H_4NO_2Cl$: C, 49.50; H, 2.36. Found: C, 49.37; H, 2.10.

5-Carboxybenzoxazolinone. A modification of the method of Cavill (1945) was used to prepare methyl 3-nitro-4-hydroxybenzoate. Methyl *p*-hydroxybenzoate, 21 g. (0.138 mole), was partially dissolved in 70 ml. acetic acid. This mixture and a solution of sodium nitrite, 5.4 g. in 60 ml. water, were slowly added to a well-stirred solution of nitric acid, 42 ml. of 15 N acid diluted to 160 ml., using a dropping funnel for the sodium nitrite solution. The mixture was heated for 1 hour while being stirred. The reaction mixture was then poured onto 250 g. of ice, and the yellow solid which formed was filtered in vacuo. The product was crystallized from aqueous acetic acid to give yellow needles, m.p. 72°-72° C. (lit. 74°) in a 75 percent yield.

Methyl 3-nitro-4-hydroxybenzoate, 22 g. (0.1117 mole) was reduced to methyl 3-amino-4-hydroxybenzoate by dissolving in 150 ml. of absolute ethanol and adding 1 g. of Raney nickel catalyst. Hydrogenation at 3 atmospheres pressure was continued for 1 hour. The catalyst was removed by filtration, the solvent was removed, and the product was recrystallized from aqueous alcohol. The compound was obtained as light brown needles, m.p. 88°-89.5° C. (lit. 90°) in a yield of 76 percent.

3-Amino-4-hydroxybenzoic acid was prepared from 10 g. (0.06 mole) methyl 3-amino-4-hydroxybenzoate by refluxing in 150 ml. of 10 percent HCl for 3 hours. The solution was neutralized with dilute NaOH and made acidic (pH 6.5) with acetic acid. A brown product was obtained and was filtered in vacuo. The acid was recrystallized from water to give a 75 percent yield of yellowish-orange crystals, m.p. 202°-203° C. (lit. 201°).

To produce *5-carboxybenoxazolinone,* 3-amino-4-hydroxybenzoic acid, 2.3 g. (0.015 mole) was mixed with 1.5 g. urea and heated to 180° C. The mixture melted at 140° and the urea fusion began at 150°, and was continued for 1.5 hours. The reaction mixture was washed with aqueous acid and the residue was dissolved in absolute ethyl alcohol. The aqueous layer was extracted twice with ether. The ether extract was evaporated to dryness and the residue also dissolved in absolute alcohol. It was recrystallized from 50 percent aqueous ethanol to give white crystals which slowly decomposed at about 320° C. The nitrogen content was 7.61; the calculated value for $C_8H_5O_4N$ was 7.83.

2-Thiol-5-chlorobenzoxazole. — 2-Nitro-4-chlorophenol was reduced catalytically using platinum oxide at 2 atmospheres hydrogen pressure. The product of this reduction was 2-amino-4-chlorophenol, of which 4.9 g. (0.0308 mole) was added to a mixture of 2.3 g. KOH in 34 ml. methanol, 6.2 ml. water, and 2.6 g. carbon disulfide (Katz and Cohen 1954). The mixture was refluxed for 15 hours. The solution was cooled, treated with charcoal, and heated with 6 ml. of acetic acid. The precipitate was recrystallized from benzene to yield 3.0 g. of a solid, m.p. 275°-276° C.

2-Thiol-5-isopropylbenzoxazole.—p-Isopropylphenol was nitrated by the method of Beer et al. (1954) to give 2-nitro-4-isopropylphenol. This compound was reduced catalytically to give 2-amino-4-isopropylphenol, m.p. 142°-143° C. Into 55 ml. of methanol were placed 7.3 g. (0.048 mole) 2-amino-4-isopropylphenol, 3.6 g. potassium hydroxide, and 4.2 g. carbon disulfide. The mixture was refluxed 16 hours. The reaction mixture was neutralized with acetic acid, concentrated, and extracted with hot benzene. On recrystallization from benzene, 2.5 g. of white needles, m.p. 133°-134° C. were obtained.

2-Thiol-5-t-butylphenol.—This compound was prepared exactly as above, except *p*-t-butylphenol was used instead of *p*-isopropylphenol. The yield was 0.98 g. of fine white needles, m.p. 127°-128° C., from 6.62 g. (0.04 mole) of 2-amino-5-t-butylphenol.

Naphthoxazolinone (naphtho(4,5a)oxazole-2-one). —1-Amino-2-hydroxynaphthalene hydrochloride, 5.3 g. (0.027 mole) was fused with 2.4 g. (0.04 mole) urea. The resulting black mass was washed several times with dilute acid and the insoluble residue was dissolved in absolute ethanol and filtered. On evaporation of the alcohol, a reddish brown precipitate was obtained, which was recrystallized from 50 per cent aqueous ethanol. On decolorizing with activated charcoal and a second recrystallization, a white crystalline product was obtained, m.p. 219°-220° C.

BIOASSAY METHODS

Biological activity was assayed as inhibition of growth of newly hatched European corn borer larvae and the fungus *Penicillium chrysogenum* (NRRL 1951). Both assay techniques involved rearing the organisms on purified agar-based media to which known amounts of the test substances had been added. Growth of the insect larvae was measured in terms

of milligrams average body weight at the end of 8 to 10 days on the dietary media. Growth of the fungus was expressed as millimeters average colony diameter 72 hours after inoculation of Petri plates with a spore suspension. Biological activity of the test chemicals was measured as percent growth inhibition of the treated organisms, as based on the growth attained by untreated controls. When the $\log_{10}$ of the percentage growth inhibition was plotted against the $\log_{10}$ of the molar concentration, an approximately linear regression was obtained, from which molar concentration producing 50 percent growth inhibition could be determined (I_{50}). The activity of different compounds was compared on the basis of the I_{50} values obtained. For convenience in comparing the activity of the analogs with the activity of the parent compound of the series, Activity Coefficients were calculated as the I_{50} of the parent compound divided by the I_{50} of the analogs. Details of the other procedural aspects of the bioassay systems were published in an earlier paper (Beck and Smissman 1960).

RESULTS AND DISCUSSION

Structure and Activity of Benzoxazole Analogs.—Benzoxazole may be considered the parent compound of a series, of which benzoxazolinone is a member. A number of benzoxazole analogs were tested in which the individual substances differed in respect to substituents at position 2. Benzoxazole showed a low order of inhibitory activity against both the corn borer and *Penicillium chrysogenum* (table 1.) Substitution of methyl or chloro groups for hydrogen at position 2 increased the activity slightly. Oxygen at that position (benzoxazolinone) resulted in a nearly 10-fold increase of activity against the borer, but showed a lesser improvement in the case of the fungus. A mercapto group at position 2 (2-thiolbenzoxazole) was less effective than the oxygen analog in the case of the insect, but resulted in a great increase of activity against *Penicillium* (Activity Coefficient = 59.2).

2-Amino-5-chlorobenzoxazole has been produced commercially under the trade name Flexin (McNeil), as a muscle relaxant and a drug for the treatment of gout. In the present study, it was found to be a good growth inhibitor against both *Penicillium* and borer larvae. In the case of the latter organism, it proved to be the most effective benzoxazole derivative tested. It inhibited the growth of both insect and fungus more effectively than did 5-chlorobenzoxazolinone (table 2), indicating that the substitution of an amino group at position 2 increased the biological activity of the molecule.

o-Aminophenol is related to benzoxazole but lacks the carbon of position 2. This aminophenol was highly active against the fungus, but about half as effective as benzoxazolinone against the European corn borer. The activity of *o*-aminophenol appeared to be roughly similar to that of 2-thiolbenzoxazole.

Wahlroos and Virtanen (1958) observed that growth inhibitory activity of 6-methoxybenzoxazolinone and benzoxazolinone against *Fusarium nivale* in suspension or agar cultures declined and disappeared over a period of several days. They attributed the loss of activity to a degradation of the benzoxazolinone compounds, and were able to demonstrate the occurrence of such degradation. They postulated, however, that the benzoxazolinones were degraded to the corresponding aminophenols through oxidative destruction of the oxazole ring, but were unable to demonstrate such a chemical change. It was assumed that the conversion of benzoxazolinone compounds to aminophenols would constitute detoxication and would account for the observed loss of activity in their fungal culture media. The results of the present study clearly show that degradation to *o*-aminophenol would not constitute detoxication, but would result in the manifestation of even higher levels of growth inhibition (table 1). Similar results were obtained from experiments employing several different species of fungi of the genus *Fusarium*. Both the analytical data of Wahlroos and Virtanen (1958) and the activity data here presented make it seem unlikely that

Table 1.—Effect of benzoxazole analogs on growth of the European corn borer and *Penicillium chrysogenum*.

Compound	Effect			
	Penicillium		European corn borer	
	(I_{50})	Act. Coeff.	(I_{50})	Act. Coeff.
Benzoxazole	7.7×10^{-3}	1.00	2.4×10^{-2}	1.00
2-Chlorobenzoxazole	2.0×10^{-3}	3.79	1.2×10^{-2}	2.00
2-Methylbenzoxazole	5.9×10^{-3}	1.31	1.4×10^{-2}	1.71
2-Oxybenzoxazole (benzoxazolinone)	2.1×10^{-3}	3.67	2.5×10^{-3}	9.60
2-Thiolbenzoxazole	1.3×10^{-4}	59.2	5.1×10^{-3}	4.71
2-Amino-5-chlorobenzoxazole	4.2×10^{-4}	18.3	1.2×10^{-3}	20.0
o-Aminophenol	1.6×10^{-4}	48.1	4.9×10^{-3}	4.90

detoxication involves degradation to aminophenols. In the case of the European corn borer, *o*-aminophenol was less toxic than benzoxazolinone, but detoxication via hydroxylation at position 6 appears to be the more likely detoxication route (Bray et al. 1952, Beck 1960).

Structure and Activity of Benzoxazolinone Analogs.—A number of analogs of benzoxazolinone were assayed for growth inhibitory activity (table 2). Activity Coefficients were calculated on the basis of benzoxazolinone as the parent compound. The 6-methoxy analog is corn Resistance Factor A, and showed activity differing but little from that of benzoxazolinone. Substituents at position 6 appeared to result in slightly higher activity than did similar groups at position 5. 6-Chlorobenzoxazolinone was the most active analog in this series. Inhibitory activity of benzoxazolinone was destroyed by the addition of a highly polar group, such as amino, carboxy, or carbamindo at positions 5 or 6. Blocking the nitrogen of the oxazole ring (5-methoxy-N-acetylbenzoxazolinone and 6-methoxy-N-benzoylbenzoxazolinone) had little effect on the biological activity. A small quantity of 6-methoxy-N-methylbenzoxazolinone was supplied us by R. H. Hamilton, Department of Plant Biochemistry, Michigan State University, and its activity against the fungus was about two-thirds that of 6-methoxybenzoxazolinone. These results indicate that blocking the imido group with a stable substituent had the effect of reducing the biological activity. Growth of the European corn borer was much less sensitive to structural changes in this series of chemicals than was that of *Penicillium*. No analog tested produced growth inhibition that was dramatically higher than that caused by benzoxazolinone.

Structure and Activity of Benzothiazole Analogs.—Benzothiazole differs in structure from benzoxazole in that position 1 is occupied by sulfur rather than oxygen. Measurement of the growth inhibiting activity of benzothiazole indicated that it was a more powerful inhibitor than benzoxazole ($I_{50} = 1.1 \times 10^{-3}$ and 7.7×10^{-3}, respectively, with *Penicillium*, and 1.4×10^{-3} and 2.4×10^{-2}, respectively, with the European corn borer). The greater than 15-fold increase in borer-inhibiting properties prompted investigation of the biological activity of a number of benzothiazole analogs. In this series (table 3), benzothiazole was assigned as Activity Coefficient of 1.00, as the parent compound.

Only 2-chlorobenzothiazole caused greater borer growth inhibition than that produced by benzothiazole. In respect to the reaction of the fungus to the analogs, 2-amino-6-methylbenzothiazole was the only one more active than the parent benzothiazole.

Structure and Activity of 2-Thiolbenzoxazole Analogs.—As shown in table 1, discussed above, 2-thiolbenzoxazole was found to be a powerful inhibitor of fungal growth, showing an Activity Coefficient of 59.2 compared to the activity of benzoxazole. The activity of the 2-thiol analog against the borer was less dramatic, but was several times that of

Table 2.—Effect of benzoxazolinone analogs on growth of *Penicillium chrysogenum* and European corn borer.

Compound	Effect			
	Penicillium		European corn borer	
	(I_{50})	Act. Coeff.	(I_{50})	Act. Coeff.
Benzoxazolinone	2.1×10^{-3}M	1.00	2.5×10^{-3}M	1.00
6-Methoxybenzoxazolinone	1.8×10^{-3}	1.15	2.4×10^{-3}	1.06
5-Methoxybenzoxazolinone	1.7×10^{-3}	1.26		
6-Methylbenzoxazolinone	8.9×10^{-4}	2.35	1.9×10^{-3}	1.32
5-Methylbenzoxazolinone	8.9×10^{-4}	2.35	2.1×10^{-3}	1.19
5,6-Dimethylbenzoxazolinone	6.0×10^{-4}	3.48		
6-Chlorobenzoxazolinone	3.1×10^{-4}	6.74	1.4×10^{-3}	1.82
5-Chlorobenzoxazolinone	7.1×10^{-4}	2.94	1.9×10^{-3}	1.33
6-Aminobenzoxazolinone	N.A.*		N.A.*	
5-Aminobenzoxazolinone	N.A.*		N.A.*	
6-Nitrobenzoxazolinone	1.5×10^{-3}	1.39		
5-Nitrobenzoxazolinone	3.7×10^{-3}	0.56		
6-Carbamidobenzoxazolinone	N.A.*		N.A.*	
5-Carbamidobenzoxazolinone	N.A.*		N.A.*	
5-Carbomethoxybenzoxazolinone	1.3×10^{-3}	1.61		
5-Methoxy-N-acetylbenzoxazolinone	1.6×10^{-3}	1.35		
6-Methoxy-N-benzoylbenzoxazolinone	1.2×10^{-3}	1.70		

* N.A. = No inhibitory activity at the highest concentration tested.

Table 3.—Effect of benzothiazole analogs on growth of *Penicillium chrysogenum* and European corn borer.

Compound	Effect			
	Penicillium		European corn borer	
	(I_{50})	Act. Coeff.	(I_{50})	Act. Coeff.
Benzothiazole	1.1×10^{-3}M	1.00	1.4×10^{-3}M	1.00
2-Methylbenzothiazole	2.7×10^{-3}	0.41	2.2×10^{-3}	0.64
2-Phenylbenzothiazole	N.A.*		9.5×10^{-3}	0.15
2-Aminobenzothiazole	1.1×10^{-3}	1.00	1.3×10^{-3}	1.08
2-Amino-6-methylbenzothiazole	6.3×10^{-4}	1.75	1.9×10^{-3}	0.74
2-Chlorobenzothiazole	1.4×10^{-3}	0.79	8.3×10^{-4}	1.69

* N.A. = No inhibitory activity at the highest concentration tested.

benzoxazole. A short series of 5-substituted 2-thiolbenzoxazole analogs was tested (table 4). The three analogs all showed increased growth inhibitory activity when tested against *Penicillium*. The 5-t-butyl analog inhibited both organisms to the greatest degree. In respect to the inhibition of the growth of *Penicillium chrysogenum*, 5-t-butyl-2-thiolbenzoxazole was the most active compound tested in the entire investigation. Against the European corn borer, however, it was less effective than 6-methoxybenzoxazolinone.

Structure and Activity of para-substituted Phenols.—The experimental results thus far discussed led to the hypothesis that the inhibition of *Penicillium* growth was partially dependent upon lipid-solubilizing substituents on the phenol nucleus. The data did not suggest such a structure-activity relationship in respect to borer growth inhibition, however. The postulated relationships were tested by measuring the activity of a number of para-substituted phenolics (table 5).

The experimental results tended to support the hypothesis. With the fungus as the test organism, the lowest activity was observed in *p*-carboxylphenol and the highest in *p*-phenylphenol. The compounds tested against the European corn borer were without inhibitory activity.

The I_{50} of *p*-t-butylphenol against *Penicillium* was about 10 times larger than that of a 2-thiol-5-t-butylbenzoxazole. Orthoaminophenol was found to be a powerful inhibitor of *Penicillium* growth, whereas p-aminophenol showed very low inhibitory activity. These results suggested that a nitrogen substituent adjacent to an oxygen or hydroxyl group might play an important role in the biological activity.

Structure and Activity of Substituted Monophenols.—The effects of a number of analogs of *o*-aminophenol on the growth of the insect and fungus are shown in table 6. The compounds were of comparatively low activity against the European corn borer. Against *Penicillium chrysogenum*, *o*-aminophenol and *o*-nitrophenol displayed approximately equal activity. Biological activity of 2-amino analogs was greatly reduced by the introduction of chloro groups at positions 4 and 5, with the 5-chloro showing about twice the activity of the 4-chloro. The activity of 2-amino-4-chlorophenol was nearly identical to that of 5-chlorobenzoxazolinone, and that of 2-amino-5-chlorophenol was nearly equal to that of 6-chlorobenzoxazolinone. It should be noted that positions 4 and 5 in the aminophenols are comparable to positions 5 and 6, respectively, in benzoxazole analogs.

A nitro group at position 2 in 4 and 5 chloro-substituted phenols resulted in much greater activity than was observed in the corresponding 2-amino analogs. The activity of 2-nitro-5-methoxyphenol was virtually identical to that of *o*-nitrophenol. 2-Nitro-5-chlorophenol was the most powerful fungal growth inhibitor tested in this series.

It does not appear that the effects of chemical con-

Table 4.—Effect of 2-thiolbenzoxazole analogs on growth of *Penicillium chrysogenum* and European corn borer.

Compound	Effect			
	Penicillium		European corn borer	
	(I_{50})	Act. Coeff.	(I_{50})	Act. Coeff.
2-Thiolbenzoxazole	1.3×10^{-4}M	1.00	5.1×10^{-3}M	1.00
2-Thiol-5-chlorobenzoxazole	6.0×10^{-5}	2.17	5.7×10^{-3}	0.89
2-Thiol-5-isopropyl benzoxazole	4.6×10^{-5}	2.83		
2-Thiol-5-*t*-butyl benzoxazole	3.7×10^{-5}	3.51	2.9×10^{-3}	1.76

Table 5.—Effect of para-substituted phenols on the growth of European corn borer and *Penicillium chrysogenum*.

Compound	Growth inhibition			
	Penicillium		European corn borer	
	(I_{50})	Act. Coeff.	(I_{50})	Act. Coeff.
Phenol	8.5×10^{-4}	1.00	1.8×10^{-2}	1.00
p-Carboxyphenol	N.A.*	0	N.A.*	0
p-Aminophenol	8.4×10^{-3}	0.10		
p-Methoxyphenol	2.6×10^{-3}	0.33	N.A.*	0
p-Ethylphenol	8.2×10^{-4}	1.04		
p-Isopropylphenol	6.1×10^{-4}	1.39		
p-*t*-Butylphenol	3.3×10^{-4}	2.58		
p-*n*-Hexylphenol	1.3×10^{-4}	6.64	N.A.*	0
p-Phenylphenol	7.0×10^{-5}	12.14	N.A.*	0

* N.A. = No inhibitory activity at highest concentration tested.

figuration may be explained on basis of electronic phenomena. On the basis of electron positions and influences, *o*-aminophenol and *o*-nitrophenol should have shown dissimilar properties. Chloro substituents at positions 4 and 5 should also have had dissimilar effects. The biological activity of these phenolics may depend, in part, on the structural features of the moiety with which they combine in the organisms; and in the absence of information on the combining system(s), detailed interpretation of the experimental results is not possible.

Structural and Activity of Analogs Miscellaneously Related to Benzoxazolinone.—Three compounds bearing some structural similarity to benzoxazolinone were assayed for growth inhibitory activity (table 7). Naphthoxazolinone differs from benzoxazolinone in that a naphthaloid rather than a benzenoid nucleus is attached to the oxazole ring. Its activity against *Penicillium* was considerably greater than that of benzoxazolinone, possibly because of greater lipid solubility. Against the insect, the activity of naphthoxazolinone was almost identical to that of benzoxazolinone.

The indole nucleus differs from that of benzoxazole by the absence of oxygen from the five-membered ring. The auxin indole-3-acetic acid was without activity. Indole-3-acetonitrile was about equal to benzoxazolinone against the corn borer, but more effective against the fungus.

CONCLUSIONS ON STRUCTURE-ACTIVITY RELATIONSHIPS

In regard to the inhibition of European corn borer growth, none of the chemicals tested proved to be outstandingly more effective than benzoxazolinone. The introduction of chloro groups at positions 5 and 6 of the benzoxazolinone nucleus increased inhibitory activity, but the effect was relatively minor. Similarly, the substitution of sulfur for oxygen at position 1 (creating a benzothiazole) increased the growth-inhibiting effects significantly, but the I_{50} values were of the same order of magnitude. The

Table 6.—Effect of substituted monophenols on growth of *Penicillium chrysogenum* and European corn borer.

Compound	Effect			
	Penicillium		European corn borer	
	(I_{50})	Act. Coeff.	(I_{50})	Act. Coeff.
o-Aminophenol	1.6×10^{-4}M	1.00	4.9×10^{-3}M	1.00
o-Nitrophenol	1.8×10^{-4}	0.89	1.2×10^{-2}	0.42
2-Amino-4-chlorophenol	7.2×10^{-4}	0.22	6.5×10^{-3}	0.75
2-Nitro-4-chlorophenol	4.3×10^{-5}	3.72		
2-Amino-5-chlorophenol	3.7×10^{-4}	0.43		
2-Nitro-5-chlorophenol	3.9×10^{-5}	4.10		
2-Nitro-5-methoxyphenol	1.8×10^{-4}	0.89	1.2×10^{-2}	0.42
2,4-Dinitrophenol	3.0×10^{-4}	0.53	3.2×10^{-3}	1.53
5-Methoxy-2,4-dinitrophenol	1.4×10^{-4}	1.14	$>7 \times 10^{-2}$	

Table 7.—Effect of benzoxazolinone analogs on growth of *Penicillium chrysogenum* and European corn borer.

Compound	Effect			
	Penicillium		European corn borer	
	(I_{50})	Act. Coeff.	(I_{50})	Act. Coeff.
Benzoxazolinone	2.1×10^{-3}M	1.00	2.5×10^{-3}M	1.00
Naphthoxazolinone	2.5×10^{-4}	8.36	2.6×10^{-3}	0.96
Indole-3-acetic acid	N.A.*		N.A.*	
Indole-3-acetonitrile	5.8×10^{-4}	3.62	3.0×10^{-3}	0.83

* N.A. = No inhibitory activity at highest concentration tested.

most effective compound tested was 2-chlorobenzothiazole; the I_{50} obtained was 8.3×10^{-4}. Compared to the activity of benzoxazolinone, the borer Activity Coefficient of 2-chlorobenzothiazole was 3.07.

The experimental data indicated that the physiological effect on the corn borer was associated with the configuration of the benzoxazolinone molecule, and that departures from that structure generally had the effect of reducing the biological activity, with the minor exceptions noted above. Phenolic compounds were relatively ineffective as borer growth inhibitors, indicating that the oxazole ring is involved in the biological activity. The experiments conducted did not provide any direct clue to the biochemical action of benzoxazolinones. The clinical use of benzoxazolinones in the treatment of gout symptoms is suggestive that purine metabolism may be affected by compounds of this type. This aspect is being investigated currently.

The biological activity of benzoxazoles, benzothiazoles, and phenols appeared to be closely related in the case of their inhibitory effects on *Penicillium chrysogenum*. The fungus was much more sensitive to structural differences than was the insect, and the range of I_{50} values was much larger (as low as 3.7×10^{-5}). Growth inhibition of the fungus was associated with at least two structural features of the molecule: (1) lipid solubility, shown as increased activity in the presence of lipid-solubilizing substituents on the phenyl ring; and (2) the presence of a nitrogen (nitro or amino) group ortho to a phenolic hydroxyl, as indicated by the observation that ortho-nitro and ortho-amino phenolics were of much greater activity than were benzoxazole derivatives. The latter characteristic was interpreted as indicating that the oxazole ring reduced the molecule's effectiveness as an inhibitor of fungal growth.

There was some evidence that the 2-thiol group effectively destroyed the biological influence of the oxazole ring, as the I_{50} for *o*-aminophenol was nearly identical to that for 2-thiolbenzoxazole in the case of both assay organisms. In the case of *Penicillium*, the 2-thiol substitution and the absence of an oxazole ring both had the effect of increasing the biological activity as compared to that displayed by benzoxazolinone; whereas exactly the opposite effects were observed when activity was measured in terms of inhibition of European corn borer growth.

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The Influence of Plant Spatial Patterns on Insect Populations[1]

DAVID PIMENTEL[2]

Department of Entomology, Cornell University, Ithaca, N. Y.

ABSTRACT

Three different densities of planting were employed in this investigation of the influence of plant spatial patterns on the animal community associated with *Brassica oleracea* L. (Cruciferae). Plants were spaced at 6 inches, 18 inches, and 9 feet in rows that were, respectively, 6 inches, 36 inches, and 9 feet apart, so that on equivalent areas of 7,500 square feet there were totals of 30,600, 1,768, and 80 plants, respectively. An inverse relationship was found between plant density and the total number of animal taxa present in the various plantings. The proportion of herbivore taxa to carnivore taxa was greatest in the dense planting, least in the dispersed one, but the density of many herbivores in the sparse and dispersed plantings was more than five times that in the dense planting. Plant survival in nature often depends upon a spatial pattern of dense design.

The inaccessibility of plants in space and time may limit the numbers of animals (Andrewartha and Birch 1954). These investigators implied that spatial distribution, and a shortage of time relative to the animal's capacity for searching and finding food, are important in limiting animal populations. Although this proposition is correct, it is only one aspect of the dynamic situation. Lebedev (1924) reported that dense planting was desirable because it reduced flea beetle (*Phyllotreta* spp.) numbers on turnips. Plants in dense stands existing in time play a significant role in the ecology of animal populations, because plants are both food and a part of the animal's physical environment. The influence of plant spatial patterns in dense design on animal populations was the focus of this investigation.

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METHODS

The design of the experiments was based on the following hypotheses. First, plants which grow densely in space may sacrifice a number of their population without affecting their survival. Second, as the vegetation that is available increases per unit time to the herbivore population, less destruction is caused by the herbivore to the plant population.

The animal community selected for the study was that associated with *Brassica oleracea* L. Members of this community are abundant in numbers, both of species and of individuals (Pimentel, unpublished data), and occur on both natural and cultivated Cruciferae in the region of Ithaca, New York (Hering 1932). The biennial growth characteristics of *B. oleracea* were of importance, providing stability to the plant factor in the test environment, and the investigator's familiarity with this community was of equal significance.

The varieties of *B. oleracea* used in 1957 were cabbage, collards, broccoli, Brussels sprouts, and kale. These differed little in their attractiveness to animals (Pimentel, unpublished data), but collards and broccoli blossomed readily and provided nectar for those individuals (such as adult Lepidoptera and Hymenoptera) that required it, and the broad, flat leaves of collards facilitated insect counting. Hence collards alone were used in 1958.

Three plant spacings were used in the design. In the "dense planting" the plants were spaced 6 inches in the row and 6 inches between the rows;

[1] Accepted for publication June 17, 1960.
[2] Associate Professor of Insect Ecology.

in the "sparse plantings" the standard cultivation spacing, 18 inches in the row and 36 inches between rows, was used; and in the "dispersed plantings," used only in 1958, the plants were spaced 9 feet in the row and 9 feet between rows. On equivalent areas of 7,500 square feet, totals of 30,600, 1,768, and 80 plants stood in the dense, sparse, and dispersed plantings, respectively. Plots 75x100 feet each were employed in the dense and the sparse plantings, but a larger plot, 300x900 feet, was used for the dispersed planting so as to hold a satisfactory number of plants. The dense- and sparse-planting plots were laid out lengthwise in an east-west direction, with 50 feet separating them to reduce the chance that animals would be blown from one plot to the other. The prevailing winds were north-northwesterly. To isolate the plots further, a fence 5 feet high was constructed between them, and sunflower plants and weeds were encouraged on the fertilized ground separating them. This arrangement proved satisfactory, as will be apparent from the results. The dispersed-planting plot was located about 800 feet east of the other plots.

All the plots were fertilized at a rate of 2,000 pounds of 5-10-5 mixture per acre. Lime was applied at a similar rate. All plots were maintained weed-free.

During the 1957 season the plants germinated from seed set in the field, and were thinned later to the desired spatial pattern. In 1958 the plants in the dense and the sparse plantings were handled similarly, but those for the dispersed planting were started in a cold frame and then were set out in the field. Because of the better temperature and moisture conditions initially, the latter plants grew faster and were larger early in the season than those seeded directly in the test plots.

Starting June 18, 1957, and weekly thereafter for a 12-week period, 100 plants were sampled in each test plot, but not the same 100 plants each week. In 1958, weekly checks began June 3, and continued for 15 weeks. The plants were chosen systematically —every fifth or tenth plant, depending on the total number of plants in the plot. No bias was introduced. Outer rows were not included in the sample. An estimate of the total plant-surface area was made by placing a sheet of plastic, etched with 1-inch squares, against the plant and estimating the number of square inches of surface area on the plant. Only one side of the leaf was included in the measurement. Areas estimated in this way had a measured error of no more than 10 percent.

Animal populations were sampled weekly in each plot on the same 100 plants checked for plant area. The animal species making up the community associated with *B. oleracea*, though varied, consisted principally of insects. In extensive preliminary work, a thorough study was made of the relationships and interactions of all the animals of the test community, and this information was available to the investigator for reference (Pimentel, unpublished data).

In estimating the number of individuals of any taxon, its life-history stage which provided the most accurate estimate of density was selected. A list follows of the types found, with the methods by which their densities were determined.

Lepidoptera.—All members of this order were counted in the last larval instar. As the larvae were counted, they were collected and placed in separately labeled vials, then taken to the laboratory and held to determine rates of parasitism and disease. Pupal stages were not counted, but were collected and held to detect any parasites not common to them and to larvae.

Homoptera.—Members of this order were counted in the adult stage. Aphidae that were parasitized and attached to the plant were counted as parasitized, but those from which parasites had emerged were not counted. Samples of the parasitized aphids were taken to the laboratory and held to determine the parasites and their ratios.

Hemiptera.—Members of this order were counted in the adult stage. Parasitism was recorded when observed.

Coleoptera.—Members of this order were counted in the adult stage. Last-stage larvae were collected and held for emergence of parasites.

Diptera.—*Liriomyza, Scaptomyza,* and *Aphidoletes* were counted in the third-larval instar. Pupae were held for parasite emergence. Syrphidae were counted in the pupal stage, and were held to determine parasites and rates of parasitism. All other Diptera were counted in the adult stage, and larvae, when seen, were collected to determine parasitism rates.

Hymenoptera.—Except for the species of Formicidae, members of this order were counted as so many parasitic attacks upon their hosts—that is, 10 infected hosts were recorded as 10 parasites of some species. Hosts were collected in the field, and held for parasite emergence.

Neuroptera.—Members of this order were counted in the last larval stage, and were held in the laboratory to determine species and parasitism rates.

Arachnida.—All stages of both Araneae and Acarina were counted, and rates of parasitism determined when possible.

Other Macroscopic Animals.—These were counted in the adult stage, and parasitism was determined when possible.

Microorganisms.—These were counted as so many animal-host infections.

In sampling the populations, it was difficult to distinguish the differences between some species, genera, and families. Therefore, such organisms were classed into "taxa" which could be determined accurately in the field, and the taxa in all the test communities were handled similarly. The method of grouping for counting purposes reduced the categories of taxa to about one-fourth, when compared with the initial preliminary survey. The taxa and their ecological roles will be reported in a later paper.

RESULTS

In 1957, a total of 20 taxa were found associated with *B. oleracea* grown in the dense planting, 39 taxa in the sparse planting (table 1). In 1958, the differences between the dense and the sparse plantings, although significant (39 and 50 taxa, respectively), were not as marked as in the 1957 season (table 1). Further support of the trend—an inverse relationship between plant density and number of taxa present—was found upon comparison of the 1958 sparse and dispersed plantings, though the difference (50 and 54 taxa, respectively) was not great.

Table 1.—Number of taxa found in the dense and in the sparse planting of *Brassica oleracea* in 1957 only, in 1958 only, and those common to both years.

Classes	Dense planting			Sparse planting		
	In 1957 only	Common to both years	In 1958 only	In 1957 only	Common to both years	In 1958 only
Herbivores	2	13	11	3	16	12
Parasites	0	3	7	3	9	2
Predators	0	2	3	5	3	8
Totals	2	18	21	11	28	22

Differences existed in the proportions of herbivores, parasites, and predators in the various plantings (table 2). There were fewer carnivore taxa, relative to herbivores, in the dense than in either the sparse or the dispersed plantings. That the sparse planting had a lower proportion of carnivores than the dispersed supports the general trend—that as the density of plants decreased, the proportion of carnivores to herbivores increased. Conditions for carnivores were more favorable in the less dense stands, where the herbivores were more abundant.

Table 2.—The number of taxa found only in each type of planting compared with those common to the contrasted plantings, during the 1957 and 1958 seasons.

Classes	Taxa common to both	Dense only	Sparse only	Dispersed only
1957: Dense planting vs. sparse planting				
Herbivores	15	0	4	—
Parasites	3	0	9	—
Predators	2	0	6	—
Totals	20	0	19	—
1958: Dense planting vs. sparse planting				
Herbivores	22	2	6	—
Parasites	9	1	2	—
Predators	5	0	6	—
Totals	36	3	14	—
1958: Sparse planting vs. dispersed planting				
Herbivores	26	–	2	3
Parasites	9	–	2	4
Predators	11	–	0	1
Totals	46	–	4	8
1958: Dense planting vs. dispersed planting				
Herbivores	23	1	—	6
Parasites	10	0	—	3
Predators	5	0	—	7
Totals	38	1	—	16

Most of the species taken in both the dense and sparse plantings in 1957 were also present in 1958, yet from the first year to the second the total number of taxa found increased 95% in the dense and 28% in the sparse plantings (table 1). This increase consisted of an equal number of carnivores and herbivores. Although the plantings were changed from a few collards plus other varieties of *B. oleracea* in 1957 to collards alone in 1958, this was not considered responsible for the changes noted in the number of taxa. The movement of transient carnivores and general-feeding herbivores, plus variation in weather, are valid explanations of the changes. Weather was the more important of these. Appreciably lower temperatures in 1958 were accompanied by a 42% increase in rainfall (table 3). Such conditions would be expected to cause significant changes in the general makeup of the community.

Table 3.—Mean monthly temperatures and rainfall for the experimental periods in 1957 and 1958.

Year	May	June	July	Aug.	Sept.
	Monthly temperatures (° F.)				
1957	53.9	67.4	67.4	63.6	60.0
1958	52.4	59.2	68.1	66.6	59.2
	Monthly rainfall (inches)				
1957	3.30	3.09	5.55	1.43	3.15
1958	3.37	5.94	5.23	4.83	4.17

Significant changes in species density occurred with changes in plant density. Because of limited space, only the following categories have been chosen for consideration: *Myzus persicae* (Sulz.), other aphids, lepidopterons, flea beetles, miscellaneous herbivores, parasites, and predators. A list of all the taxa and detailed weekly population data for each one are on file in the Comstock Memorial Library at Cornell University, and a microfilm of these may be obtained on request. The presentation of population statistics is complicated by the variation in plant size resulting from competition (Pimentel et al., unpublished data). The mature plants in the dispersed plantings were the largest, those in the dense planting the smallest, but the latter grew faster in their early stages and thus were larger for the first 5 or 6 weeks. With such pronounced differences in size, certain qualitative differences in nutritional content might be expected. No allowances could be made for such qualitative changes, but the quantitative differences in size of the plants were considered. To do this, the measurements of the surface area recorded for each plant were used in adjusting animal densities, which were made equivalent on a constant basis of density per unit plant area of 20,000 square inches.

In general, more herbivores were present per unit plant area in the sparse and the dispersed than in

the dense plantings. The important exception to this trend was the aphid herbivore, *M. persicae*. In 1957 there was no significant difference in its populations between the dense and the sparse plantings, which averaged 20.1 and 21.5 aphids, respectively, per 20,000 square inches of leaf surface for the 12-week season (fig. 1). During 1958, however, *M. persicae* was much more abundant on the plants in the dense planting than on those in either the sparse or the dispersed ones, with averages of 57.3, 21.4 and 20.2, respectively (fig. 2). *M. persicae* was most abundant during the cool spring and fall, with peak densities occurring in the fall.

Aphid species other than *M. persicae* were generally more abundant in the sparse and the dispersed plantings than in the dense one. In 1957, aphid density averaged 141.2 in the sparse and only 25.0 in the dense planting (fig. 3). The trend in 1958 was similar, with aphids averaging 34.5 in the sparse and only 4.4 in the dense planting, but with an average of 23.4 in the dispersed one (fig. 4). It should be noted, however, that their density in the early part of the season was low in the dispersed as compared to that in the sparse planting, but the aphids became abundant in the former and scarce in the latter as the season progressed.

In 1957, the Lepidoptera were significantly more abundant in the sparse than in the dense planting, averaging 12.8 and 2.1, respectively (fig. 5). This trend was substantiated in 1958, with corresponding averages of 16.3 and 3.4, respectively (fig. 6). Initially their density in the despersed plot was low, but became higher than that in the sparse plot by the end of the season (fig. 6).

Flea beetles were significantly more abundant in the sparse than in the dense planting during 1957 (fig. 7) and again in 1958 (fig. 8). Their respective averages were 1,107.6 and 185.2 for 1957, and 1,007.9 and 120.8 for 1958. They were less abundant in the dispersed than in the sparse planting during the early half of the 1958 season, but reversed this relationship late in the season.

Because the miscellaneous herbivore category is composed of numerous species, the trends in population density are not clear in figures 9 and 10. An excessively large number of Collembola were present on the plants in the dense planting at the start of the 1957 season, equivalent to 60,872 individuals per unit plant area of 20,000 square inches. Growth of these animals was favored by the higher moisture levels here, as compared with those in the sparse planting (Pimentel et al., unpublished data). The dense population of Collembola did not cause any noticeable injury to the plants.

Parasites were generally more numerous whereever herbivores were abundant. In 1957, parasites averaged 27.8 in the sparse, 1.8 in the dense planting (fig. 11). A similar trend was noted in 1958, with averages of 14.1 and 3.7, respectively, in these plantings, and an average of 8.6 in the dispersed planting (fig. 12). In this instance the trend toward high density late in the season in the dispersed plot did not develop, probably because lack of time prevented increase of parasitism on the high population of herbivores.

Predators varied in density. For 1957, little significance can be attributed to averages of 8.9 and 8.2 predators in the sparse and the dense planting, respectively (fig. 13). In 1958, averages of 13.0, 5.1, and 3.9 were recorded for the dispersed, sparse, and dense plantings, respectively. Possibly the open conditions found in the dispersed planting favored the activities of the predators. Spiders made up the largest portion of the predaceous fauna, and one reason for their large number was that they were counted in all stages, as often the nymphal stadia could not be distinguished from adults in the field. However, this trend would still have been evident if only adults had been counted.

Accurate estimates of birds could not be made, and their role as predators in this community was not assessed. *Melospiza melodia* (Wilson), *Spizella passerina* (Bechstein), and *Passer domesticus* (L.) seemed to prey mostly on the large lepidopteran larvae such as *Pieris napae* (L.) and *Trichoplusia ni* (Hbn.).

DISCUSSION AND CONCLUSIONS

The significance of this study rests in evaluating the changes both in species and in density of the members of the community associated with *Brassica oleracea* in various spatial patterns. The reduced number of herbivorous species in the dense planting is thought to result from the effects of minimal populations (Allee et al. 1949)—that is, because of low population densities some types had difficulty in maintaining their numbers. Without certain herbivorous types, dependent parasites and predators did not exist.

The total number of herbivores *per unit plant area* was greater in both the dispersed and the sparse plantings than in the dense one. This was due to the significantly greater quantity of food supplied per unit time in the dense planting. The increased plant material available here, as compared with the other plantings, resulted in a decrease in herbivore density because the latter was measured relative to plant-surface area.

This kind of comparison is valid if all the plantings were suitable for the animals; however, some differences existed both in food quality and in microclimatic conditions caused by the dense plant stands. Fagan et al. (1943) found that in crowded *B. oleracea* protein levels were lower and fiber content higher. Increased moisture and cooler temperature conditions occur in dense plantings of *B. oleracea* (Pimentel et al., unpublished data).

These changes, however, were not considered important limiting factors because, on a per-unit area of soil surface basis, more organisms were present in the dense planting than in the sparse and the dispersed plantings. Counts made the 15th week of the 1958 season and adjusted for equivalent areas of

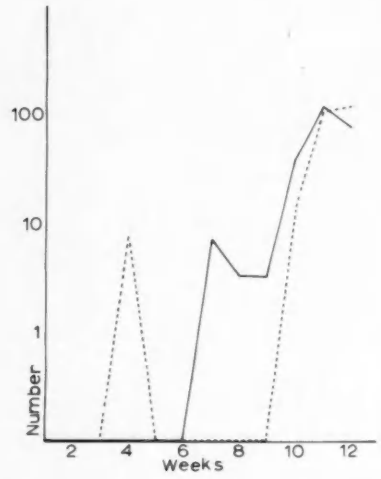

Figure 1. The number (log) of M. persicae per unit plant area in the dense (- - - - - -) and sparse (———) plantings during 1957.

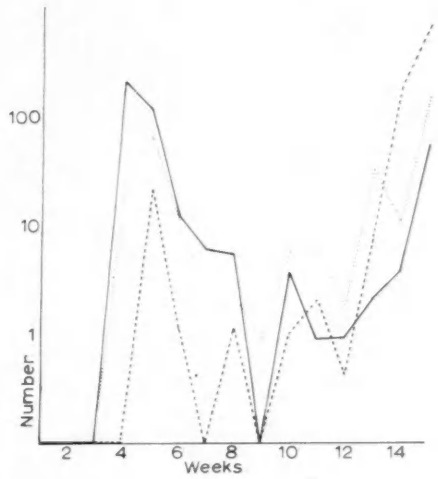

Figure 2. The number (log) of M. persicae per unit plant area in the dense (·····), sparse (———), and dispersed (— — —) plantings during 1958.

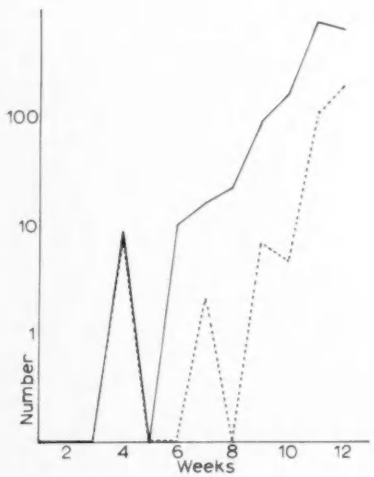

Figure 3. The number (log) of aphids other than M. persicae per unit plant area in the dense (·····) and sparse (———) plantings during 1957.

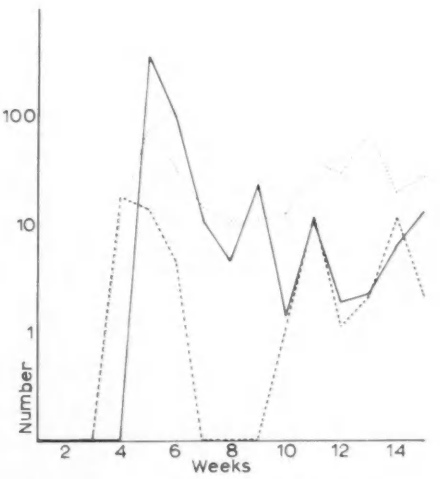

Figure 4. The number (log) of aphids other than M. persicae per unit plant area in the dense (·····), sparse (———), and dispersed (— — —) plantings during 1958.

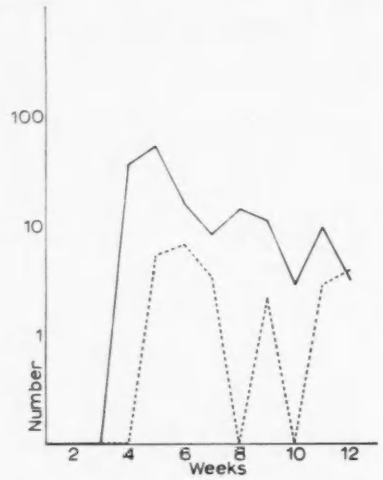

Figure 5. The number (log) of Lepidoptera per unit plant area in the dense (--------) and sparse (———) plantings during 1957.

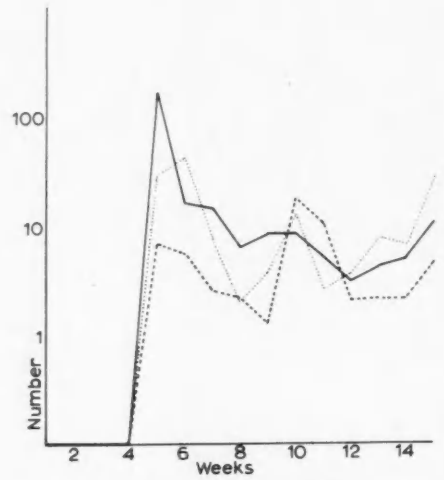

Figure 6. The number (log) of Lepidoptera per unit plant area in the dense (--------), sparse (———), and dispersed (·····) plantings during 1958.

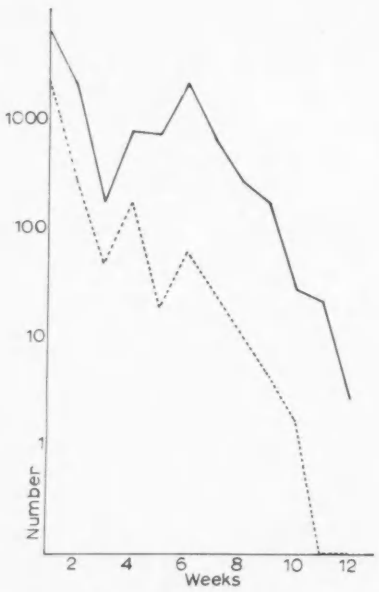

Figure 7. The number (log) of the flea beetles per unit plant area in the dense (--------) and sparse (———) plantings during 1957.

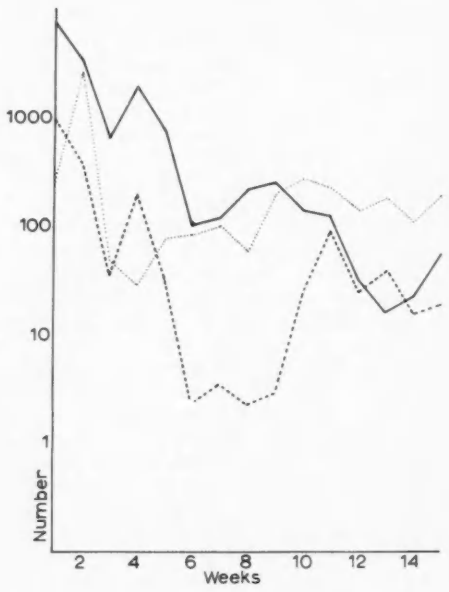

Figure 8. The number (log) of the flea beetles per unit plant area in the dense (--------), sparse (———), and dispersed (·····) planting during 1958.

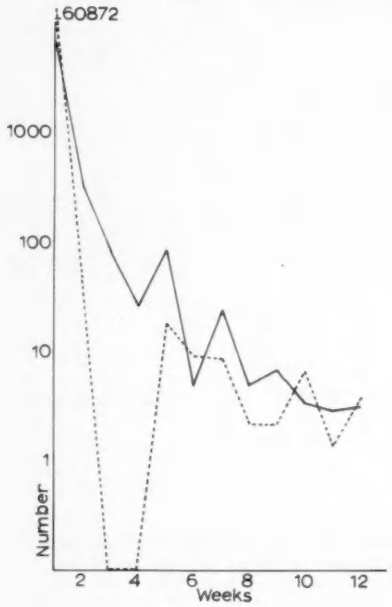

Figure 9. The number (log) of miscellaneous herbivores per unit plant area in the dense (---------) and sparse (———) plantings during 1957.

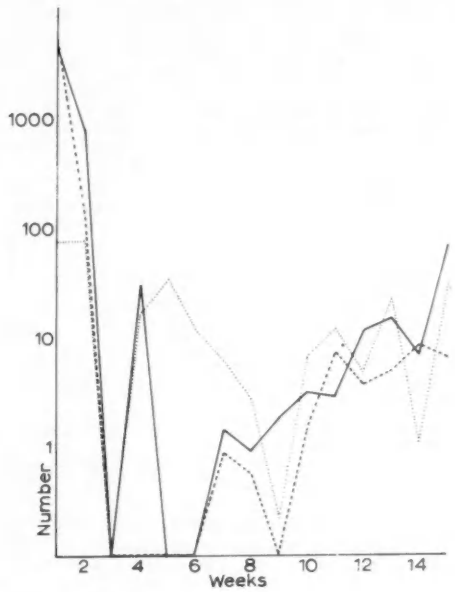

Figure 10. The number (log) of miscellaneous herbivores per unit plant area in the dense (---------), sparse (———), and dispersed (·····) plantings during 1958.

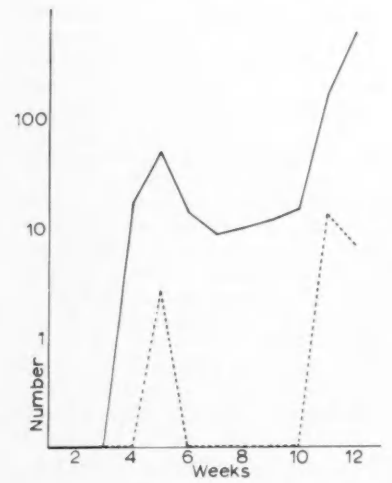

Figure 11. The number (log) of parasites per unit plant area in the dense (---------) and sparse (———) planting during 1957.

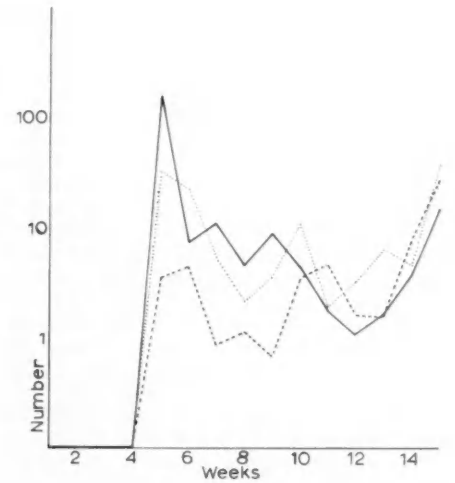

Figure 12. The number (log) of parasites per unit plant area in the dense (---------), sparse (———), and dispersed (·····) plantings during 1958.

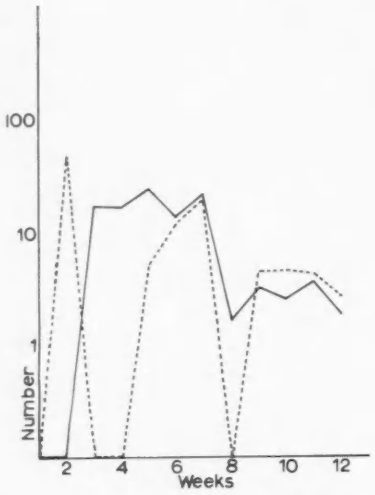

Figure 13. The number (log) of predators per unit plant area in the dense (·····) and sparse (———) plantings during 1957.

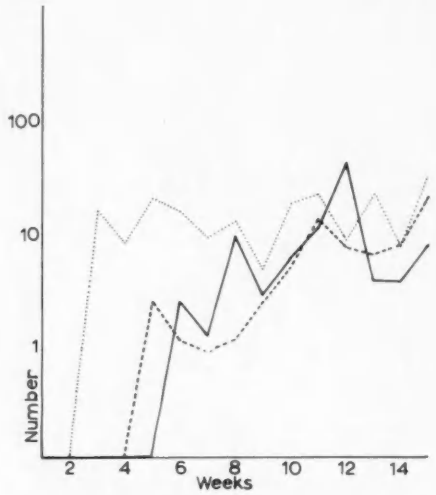

Figure 14. The number (log) of predators per unit plant area in the dense (- - - - - -), sparse (———), and dispersed (·····) plantings during 1958.

7,500 square feet of soil surface gave totals of 563,040, 29,702, and 4,792 organisms in the dense, sparse, and dispersed plantings, respectively. Thus, relative to soil-surface area, far more individuals were present in the dense than in the sparse or the dispersed planting. On the basis of plant-surface area, however, fewer individuals were present in the dense than in the other plantings. The 30,600 plants in the dense planting supplied the herbivores with the largest quantity of food in the shortest time, as compared with either the 1,768 plants in the sparse planting or 80 plants in an equivalent area in the dispersed planting. In fact, the large amount of vegetation available in the dense planting reduced the destructive effect of the large herbivore populations.

In the dispersed planting, herbivores were generally few in number early in the season and became abundant per plant area as the season progressed. In the early part of the season the plants averaging 8 square inches in size and placed 9 feet apart were beyond the use of the animals in space and time—that is, animals could not locate plants at a suitable rate to permit their making full use of them. This limited the animal population in the dispersed plot. As the plants here became larger, the animals could find them more easily; and since there were few plants, the animals congregated on them. Thus the density per unit plant area changed from one of low to one of high level.

Survival of plants in nature is related to their spatial patterns. Many plants produce an abundance of seeds. Often, as in the case of many Cruciferae, the seeds are dropped in the area immediately surrounding the plant. Early in the season, after the seeds germinate, there is nearly a solid mat of young plants. The small number of insects that have survived the rigors of winter find these dense plant stands, but destroy only a few plants, whereas the same number of insects finding fewer plants would destroy a greater proportion of the plant population. Thus animals feeding on the crowded plants help to thin out the dense seedlings, and this enables the survivors to make better growth.

Small plants provide little vegetation and are therefore more susceptible to herbivore damage. A dense stand of young plants, however, has survival value because of numbers. On equivalent areas, in the dense planting there were 30,600 plants and a total of 18,972 square inches of vegetation, as against 80 plants in the dispersed planting with a total of 212 square inches (Pimentel et al., unpublished data). The plants in the dense planting had a decided initial advantage, both in numbers and in total vegetation. As the season progressed, the total amount of vegetation increased proportionally faster in the dispersed planting, but it never reached the level of the vegetation produced in the dense planting. Late in the season the vegetation in the dense planting totaled 15,759,000 square inches, in the dispersed planting 213,000 square inches. The critical time for survival of plants in the sparse and the dispersed plantings was early in the season, when there were only a few small plants available as food for the animals. This often resulted in damage to the sparse and the dispersed plants.

Crop plants are often spaced sparsely. This, as documented in the present study, is one reason for increased insect densities, and increased crop damage.

Thus, methods of cultivation which increase the density of a stand of plants and the quantity of vegetation relative to the insect population during the growing season will appreciably aid in reducing insect damage.

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Response of the Eye Gnat *Hippelates collusor* to Light of Different Wave Lengths[1]

ROBERT W. DORNER AND MIR S. MULLA[2]

University of California Citrus Experiment Station, Riverside

ABSTRACT

The sensitivity of *Hippelates collusor* (Townsend) to light of various wave lengths was determined by two methods. One method made use of narrow-band light filters, while the other one involved the use of a spectrophotometer. With both methods similar wave lengths of maximum sensitivity (440-450 mμ) and of minimum sensitivity (above 600 mμ) were found, but the results from the two methods differed in details. These differences are explained by light-energy differences.

Numerous studies on the sensitivity of insects to light of various wave lengths have been reported in the literature, for instance, Bertholf (1931), Weiss (1946), Fingerman and Brown (1952), Autrum and Strumpf (1953), Ballard (1958), and Stermer (1959). Most insects studied so far are insensitive to red and yellow light (see Ilse 1928, Ballard 1958 for exceptions). The wave length of light causing maximum response in some insects, as in higher animals, appears to be dependent on the light-energy level and seems to shift towards shorter wave lengths as the energy level decreases (Fingerman and Brown 1952).

During a recent attempt to build a laboratory olfactometer for the testing of chemical attractants for *Hippelates gnats*, we found it necessary to investigate the response of eye gnats to light. *Hippelates* eye gnats have long been known to be positively phototropic (Hall 1932), but no investigation of the influence of the wave length of light on their phototropic response has been reported. The present paper presents our findings on the response of the eye gnat, *Hippelates collusor*, to light of different wave lengths. Since the problem was largely a practical one, our investigations were limited to wave lengths and energy levels obtainable with small fluorescent lamps and simple filters. Subsequent spectrophotometer experiments served to substantiate the general findings of the filter experiments.

MATERIALS AND METHODS

Two- to four-day-old eye gnats reared in the laboratory (Mulla and Barnes 1957) were used in the experiments. No attempt was made to separate the two sexes except where otherwise stated.

LIGHT-FILTER EXPERIMENT.—Two 15-watt Westinghouse daylight type fluorescent lamps with a suitable ballast were built into a wooden box (figure 1). The box had a horizontal slot in which Lumetron narrow-band filters (Photovolt Corporation, New York, N. Y.) could be fitted tightly. In front of the filters, two brackets held a glass tube whose outer diameter was 20 mm. The tube, closed off at both ends with cotton, contained the eye gnats whose response was to be tested. Stray light at the joints of the box and around the edges of the filters was eliminated with black tape. All tests were conducted in a photographic darkroom.

Equalizing of Light Energy.—Data on the relative distribution of energy among the different wave lengths of light emitted by fluorescent lamps were supplied by the manufacturer. From the energy emitted at each wave length (stated as a percent of an arbitrary standard) and from the light transmission of the filter at that wave length it was possible to calculate the radiant energy passing through the filter (again relative to an arbitrary standard). In order to obtain equal radiant energy at all wave lengths considered, layers of wax paper were placed in front of the filters. Since the light transmitted by

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[2] The authors wish to thank Mr. George Gale of Beckman Instruments, Incorporated, Fullerton, California, for supplying data on the DK-2 spectrophotometer.

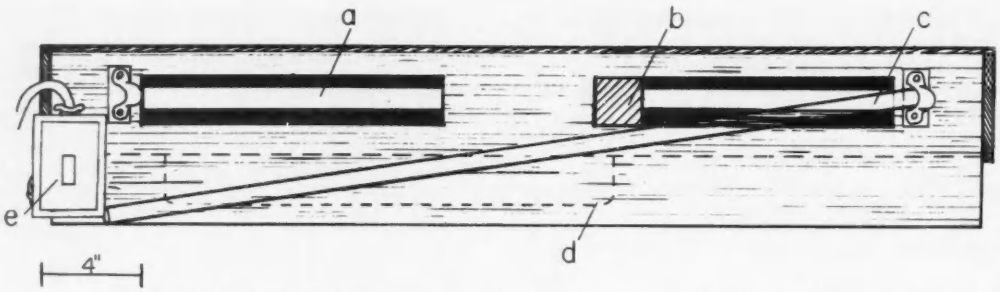

FIG. 1.—Light-filter box showing *a*, fluorescent lamp; *b*, single, narrow-band light filter, in place; *c*, glass tube for eye gnats, removed from one bracket; *d*, ballast for fluorescent lamps; and *e*, light switch.

wax paper at each wave length can be determined in a spectrophotometer, it was possible to calculate the number of layers required to reduce the radiant energy transmitted to a reasonably constant value throughout the spectrum. Of course, by this method it was not possible to equalize the energy beyond a certain limit given by the transmission of one wax paper layer at the wave length considered. When fractions of wax paper layers were required by the calculations, the fractions were rounded off to the nearest whole number. It will be seen below that the resulting energy inequalities could easily be corrected for. Furthermore, the largest error from this cause was about 0.12 optical density (OD) units—a negligible quantity in view of the much larger variations in eye-gnat response. Table 1 gives the data pertinent to these calculations.

Comparison of Attractancy of Light of Different Wave Lengths.—The light box described above was built on the assumption that it would be possible to expose eye gnats to light of equal energy at 12 different wave lengths simultaneously and that the number of eye gnats accumulated in front of each filter would be a measure of the sensitivity of the insects to the particular wave length. When this was done, however, 90% of the eye gnats congregated in front of two filters, transmitting blue and green light, thus creating the illusion that all other wave lengths were ineffective. Consequently, a more refined method of evaluating the relative attractancy of light of various wave lengths had to be developed. In the new method, eye gnats were given a choice of only two wave lengths; the others were masked with cardboard and black tape.

There are several ways of numerically evaluating the difference of attractancy between light of two wave lengths. The one chosen consists of reducing the intensity of the light of the more attractive wave length by placing additional layers of wax paper in front of the filter until the same number of gnats accumulate in front of each filter. The reduction of intensity required for even distribution of the gnats is then a measure of the relative attractancy. If the distribution of the gnats is recorded after each addi-

Table 1.—Calculations concerning the equalization of light energy in the narrow-band light-filter experiments.

Wave length, mμ	Relative energy[a] (RE)	$\text{Log}\frac{3000}{RE}$	Filter density[b] (OD_f)	$\text{Log}\frac{3000}{RE}+OD_f$ ($=A_\lambda$)	$A_{720}-A_\lambda$	OD of one wax paper layer	No. of wax paper layers[c]
720[d]	60	1.70	0.84	2.54	0.00	0.15	0.0
640	600	0.70	1.12	1.82	0.72	0.17	4.2
610	1320	0.36	1.06	1.42	1.08	0.18	6.0
590	1500	0.30	0.81	1.11	1.43	0.17	8.4
575	1700	0.25	1.33	1.58	0.96	0.19	5.1
550	1320	0.36	1.18	1.54	1.00	0.19	5.3
515	1080	0.44	1.33	1.77	0.77	0.22	3.5
465	1230	0.39	0.93	1.32	1.22	0.25	4.9
440	1500	0.30	0.94	1.24	1.30	0.20	6.5
420	780	0.59	0.88	1.47	1.07	0.21	5.1
390	150	1.30	0.81	2.11	0.43	0.23	1.9
365[d]	214	1.15	0.70	1.85	0.69	0.29	2.4

[a] Energy of the light emitted by a daylight-type fluorescent lamp in relation to an arbitrary standard. The values given here represent the area of a 30-mμ strip (approximate band width of narrow-band light filters) under the wave length vs. relative energy curve supplied by the manufacturer. In the same units a 30-mμ strip of 100% relative energy would have an area of 3000.

[b] Optical density of narrow-band light filter at its wave length of maximum transmission. Values taken from Lumetron manual.

[c] Number of wax paper layers required to reduce the light energy to a constant value at all wave lengths.

[d] Extrapolated; data available cover 390 to 700mμ.

tion of wax paper, one can plot a curve relating percent distribution to number of wax paper layers. And since the optical density of a layer of wax paper at any one wave length is known, the curve relates percent distribution to added optical density (ΔOD). These curves are sigmoid in shape with a fairly linear portion on each side of the 50-50 distribution point. Thus it is possible to interpolate between two points close to and on each side of the equal distribution point to find the ΔOD corresponding to even distribution (ΔOD_{50}) and the data reported were obtained by this method of interpolation. Physically, the ΔOD_{50} values represent the negative logarithm of the differential of light energy necessary to equally attract eye gnats with light to which they are unequally sensitive. After applying corrections for imperfect equalization of energy at some wave lengths (see above), this differential of light energy is plotted vs. wave length (see figure 2). The values are all referred to the energy value at 550 mμ arbitrarily taken as 1. No fundamental difference in results was obtained when all wave lengths were compared with 550 mμ or with 440 mμ or each with the wave length immediately above and below.

SPECTROPHOTOMETER EXPERIMENTS.—The recorder response of a Beckman DK-2 spectrophotometer (Beckman Instruments Inc., Fullerton, California) set on E (energy) is essentially the product of the energy of light supplied by the source at a particular wave length times the sensitivity of the photocell at this wave length. Thus by setting the response at 100 at the wave length of maximum sensitivity of the photomultiplier cell (340 mμ) and by manipulating the slit opening at any other wave length in such a fashion that the spectrophotometer response corresponds to the relative sensitivity of the photomultiplier tube at the new wave length, one has a light beam of constant energy throughout the spectrum.

When eye gnats are placed in a spectrophotometer cell their interference with the light beam causes a deflection of the recorder needle. The number of recorder needle movements exceeding 5% of the original reading in a 2-minute period was used as a measure of eye gnat activity. Deflections of 10% were counted as if they were two 5% deflections, etc. Various amplifier settings were used to avoid having to detect unduly small deflections. Two layers of 100-mesh brass strainer cloth were placed in front of the photocell to avoid having to work with extremely low light-energy levels.

Approximately 100 eye gnats were placed in a 10-cm. spectrophotometer cell. Between readings they were left in darkness for 5 minutes. Then they were allowed to get accustomed to light of a certain wave length for 1 minute. Finally, activity measurements were made for 2 minutes. The results are plotted graphically in figure 3.

RESULTS AND DISCUSSION

The sensitivity of eye gnats to light of different wave lengths as determined with the light-filter arrangement is shown graphically in figure 2. The graph shows the averages of three determinations and the range of the individual values. The replication of values is fair except at 575 and 590 mμ. In this region of abrupt transition from high sensitivity to low sensitivity, differential effects of light- or dark-adaptation would be most likely to have a pronounced effect. In one experiment using light-adapted eye gnats, no difference in response could be detected between complete darkness and light of 610, 640, and 720 mμ. On the other hand, eye gnats which were dark-adapted by a 1-day stay in complete darkness responded readily to light of 720-mμ wave length.

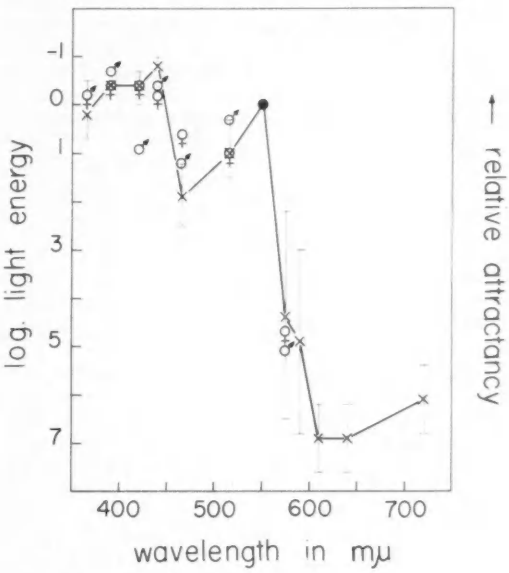

FIG. 2.—Relative attractancy to eye gnats of light of different wave lengths as determined by the light-filter box.

The ordinate represents the logarithm of the light-energy differential required to attract equal numbers of eye gnats with light of different wave lengths. The value at 550 mμ was chosen as a standard (●) (See text for details). The x points are average results from three experiments with mixed populations. The ♂ and ♀ points represent results from single experiments with all male and all female eye gnats.

In the wave length region of high light-sensitivity below 575 mμ, we consistently found two maxima of sensitivity, namely at 550 and at 440 mμ. Although two-peak light-sensitivity curves have frequently been reported before, the possibility of a heterogeneity of the eye gnat population used had to be considered. In particular, the possibility of male and female eye gnats having different wave lengths of maximum sensitivity was of interest. Single experiments with only male or only female eye gnats were therefore carried out. The results of these tests, which are also shown

in figure 2, suggest no systematic departure from the results obtained with mixed populations.

In another experiment a mixed population of eye gnats was given a choice between blue (440 mμ) and green (550 mμ) light. The ones that chose blue were collected and given the same choice again. The distribution remained roughly constant, which suggests that there are no "blue-sensitive" or "green-sensitive" eye gnats but that "blue-sensitives" are also sensitive to green.

A spectrophotometer offers a much more direct and precise method of obtaining light of various wave lengths but constant energy than a filter arrangement. The spectrophotometer method therefore supplies an independent check of the spectral sensitivity curve determined by the light-filter method. Figure 3 presents graphically the averages of three experiments and the range of the individual values. There is clearly a higher level of activity at wave lengths below 500 mμ than above even though individual values fluctuate greatly. The activity value around 600 mμ may represent the light-independent activity background although it is, of course, impossible to get an activity value for complete darkness by this method.

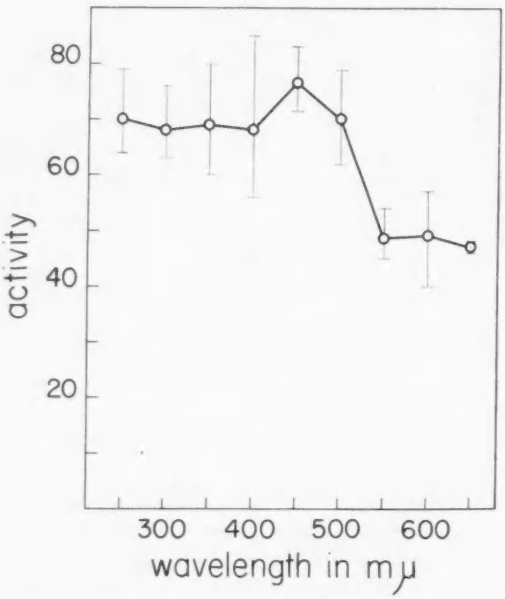

FIG. 3.—Eye gnat activity as a function of wave length of light as determined with a Beckman DK-2 spectrophotometer. (See text for details.)

The results obtained by the two methods used, while grossly similar, differ in details. Thus the sensitivity curve based on the spectrophotometer experiments has a single maximum at 440 mμ and cuts off sharply at 500 mμ, whereas the curve based on the light-filter experiments has two peaks (440 and 550 mμ) and cuts off sharply at 550 mμ. In view of the findings of Fingerman and Brown (1952) this should not be surprising. In fact, it is simply another example of the Purkinje shift of maximum sensitivity with the energy level.

Although we have no information on the exact light-energy level in our two types of experiments, it is possible to estimate the order of magnitude of this energy level from manufacturer's indications. Thus the energy flux in the light-filter experiment was of the order of 0.1 lumen; in the spectrophotometer experiment the energy flux was about one-thousandth that of the light-filter experiment. These figures are only rough estimates and the experimental conditions are not strictly comparable. It is clear, however, that the two energy levels are sufficiently different to account for the difference between the two sensitivity curves obtained.

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A Revision of the Genus *Leptoremus* (Coleoptera: Pedilidae)[1]

MOHAMMAD ABDULLAH

Department of Entomology, University of Illinois, Urbana

ABSTRACT

The genus is recharacterized on the basis of both sexes. Its type and only species, *L. argenteus* Casey, 1904, originally described only from males, is redescribed, and additional distributional records are included. Characters are given for separating *Leptoremus* from *Stereopalpus* Laferte, *Pergetus* Casey, *Mastoremus* Casey, and *Bactrocerus* LeConte.

The genus *Leptoremus* was originally described by Casey (1904, pp. 314-5) with a single included species, *L. argenteus* Casey, which automatically became its genotype. The original description of this species was based on a few male specimens only. The object of the present paper is to redescribe the genotype on the basis of both male and female characters, to present additional data on geographical distribution, and to comment on the systematic position of this monotypic genus.

I am grateful to Dr. R. B. Selander for his advice during the course of this study. For the loan of material, either from their personal collections or from collections in their care, I wish to acknowledge the assistance of Messrs. R. Snelling (S); H. B. Leech, California Academy of Sciences (CAS); T. J. Spilman, U. S. National Museum (USNM); and R. L. Wenzel (Chicago Natural History Museum (CNHM); and to Doctors G. W. Byers, University of Kansas (UK), and H. Dietrich, Cornell University (CU).

Leptoremus Casey

Medium-sized. Vestiture: body sculpture concealed by dense, decumbent hairs, short or moderate in length, without trace of erect hairs at any point. Eyes large, slightly emarginate, coarsely faceted, separated by less than half their width above. Tempora not well developed. Antennae serrate in male, filiform in female. Maxillary palpi with last segment obliquely truncate, twice as long as third. Neck broad, about half as wide as pronotum. Prothorax slightly longer than broad, narrower than head. Mesepisterna nearly meeting in front of mesosternum (fig. 5). Wing with anal cell open. Abdomen with fifth sternite entire, sixth slightly emarginate in male. Male genitalia: parameres (lateral lobes) slender, each with a row of spines on side (fig. 7); aedeagus (median lobe) sinuously serrate at apex (fig. 8). Female genitalia slender, not covered with spines, the valvifers reduced to baculi (fig. 6).

Leptoremus argenteus Casey

Leptoremus argenteus Casey, 1904: 315-6.

MALE.—Length 5.5 to 7.2 mm., width 1.6 to 2.0 mm. Color brown, with silvery pubescence on body, maculose. Head wider than prothorax, dark brown to black, covered with long, decumbent hairs; epicranial and frontoclypeal sutures obliterated, clypeolabral suture prominent. Eyes convex, dark brown. Antennae rufous, pubescent, 4.4 mm. long, serrate-compressed basally, attentuate, slender, 11-segmented; first segment twice as long as wide, second as long as wide, third to fifth serrate, in the form of right-angled triangles, longer than wide, sixth and seventh serrate, becoming longer, eighth to tenth gradually less serrate, more than three times longer than wide, last segment about as long as the preceding two combined (fig. 1). Labrum sparsely punctured, twice as broad as long, apex fringed with long hairs. Mandibles less than twice as long as broad (fig. 3). Maxillae hairy, punctate, galea much bigger than lacinia. Maxillary palpi four-segmented, first segment smallest, triangular, second longer than third, fourth largest (fig. 4). Labium small, mentum subcircular to crescentic, weakly sclerotized, ligula fringed with long hairs and punctate; labial palpi three-segmented, last segment subcircular. Prothorax brown, pubescent, apex smaller and more rounded than base. Scutellum semicircular at apex. Elytra brown, punctate, covered with silvery-white pubescence, sides parallel, becoming rounded at apex, 4.5 mm. long, 1 mm. wide. Legs brown, densely pubescent; coxae contiguous; tibiae and tarsi spinulate; tarsi 5-, 5-, 4-jointed. Abdomen covered with silvery pubescence.

FEMALE.—Length 7.2 mm., width 2.2 mm. Antennae 2.3 mm. long, smaller than in male, filiform or only feebly serrate (fig. 2). Abdomen with fifth sternite entire or only slightly emarginate.

Type Locality.—El Indio, California. The holotype is a male specimen collected by H. F. Wickham on August 19. Presumably it is in the U. S. National Museum.

Distribution.—The species is recorded from the Colorado and Sonoran deserts of the southwestern United States (fig. 9). Specimens examined are from California and Arizona, as follows. CALIFORNIA: Calexico (S, 2); Coachella Valley (UK, 1); Indio (CNHM, 5; UK, 4; USNM, 8); Jacumba (UK, 1); Thermal, Riverside Co., 100 feet below tide (CU, 12). ARIZONA: Aguila, Maricopa Co. (CU, 1); Gila Bend, 10 mi. N. (S, 3); Phoenix (CU, 1); San Luis, Yuma Co. (CAS, 1); Tempe (CU, 1); Welton, Yuma Co. (CU, 2); Welton, 1 mi. E. (CAS 1); Yuma (CU, 1). The species has been collected from May 4 to August 22.

Host Plant.—Du Bois (1956) has collected speci-

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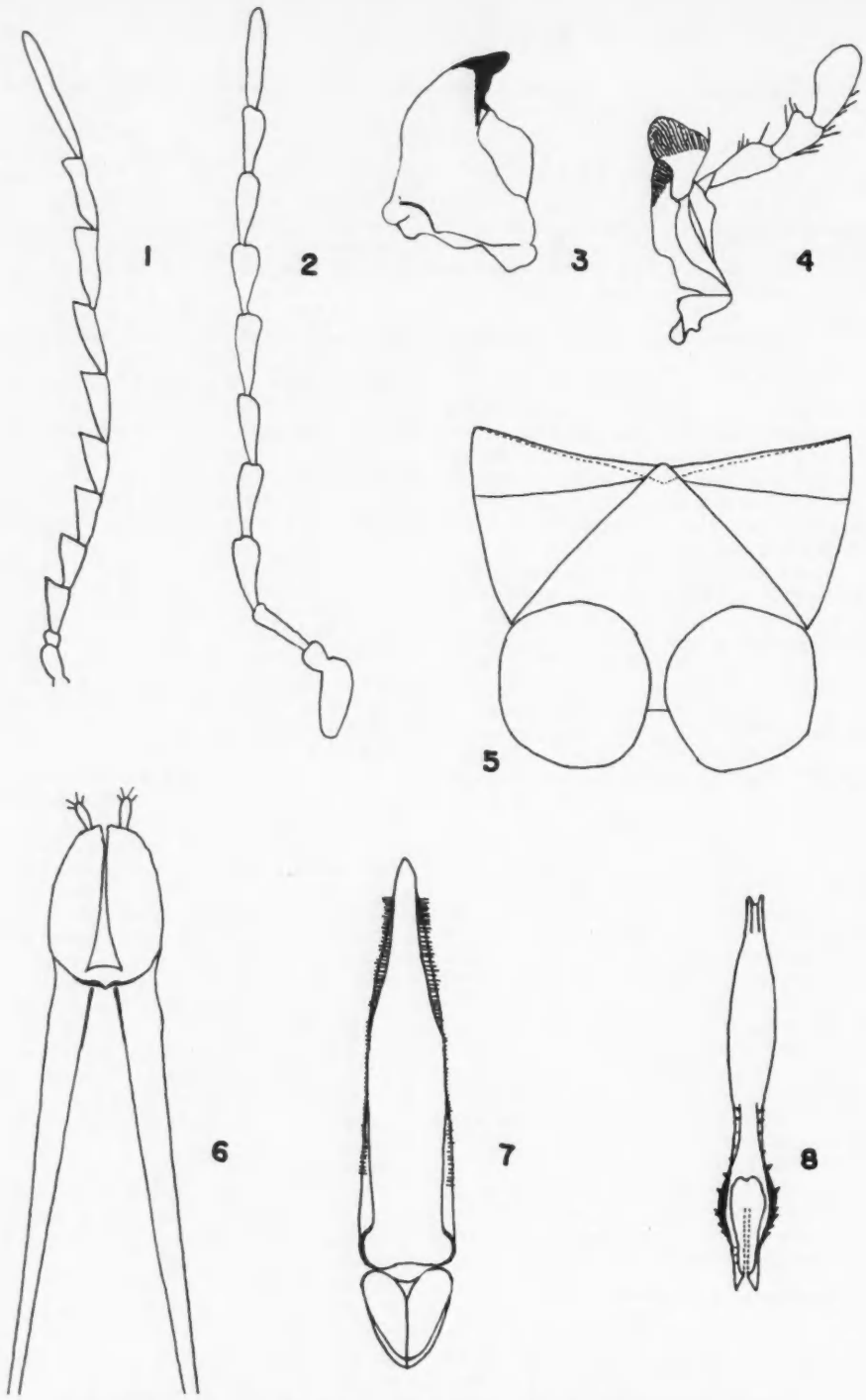

Leptoremus argenteus Casey. FIG. 1.—Antenna of male. FIG. 2.—Antenna of female. FIG. 3.—Mandible of male. FIG. 4.—Maxilla of male. FIG. 5.—Mesepisterna and mesosternum. FIG. 6.—Female genitalia, dorsal view. FIG. 7.—Tegmen of male, ventral view. FIG. 8.—Aedeagus of male, ventral view.

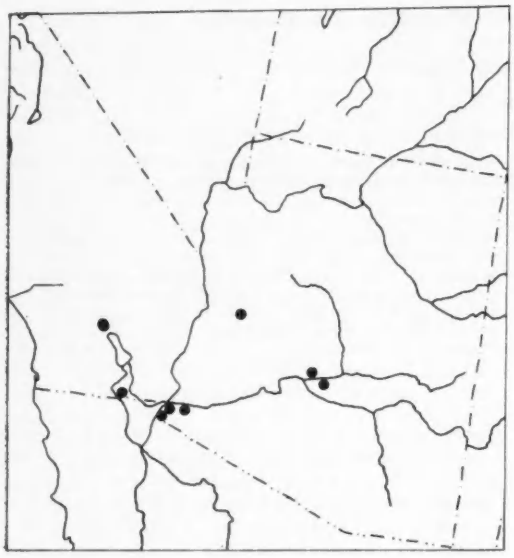

FIG. 9.—Distribution of *Leptoremus argenteus* Casey.

mens by beating *Prosopis* sp. near San Luis, Arizona.

COMPARISON WITH RELATED NORTH AMERICAN GENERA

Leptoremus is distinguished from all other described North American genera of Pedilidae by the following combinations of characters: tarsal claws simple, nonappendiculate; neck wide; eyes large, slightly emarginate, and coarsely faceted; tempora subobsolete; maxillary palpi with fourth segment obliquely truncate and twice as long as third. There are also certain differences in the characters of the male and female genitalia.

The genera with which *Leptoremus* is most likely to be confused are *Stereopalpus, Pergetus* (=*Eurygenius*), *Mastoremus,* and *Bactrocerus.* These genera are compared with *Leptoremus* in the paragraphs which follow. The distinctions between *Leptoremus* and the remaining genera of pedilid beetles will be considered in detail in a study of the classification and phylogeny of the Pedilidae now in progress.

Stereopalpus Laferte differs from *Leptoremus* by the following characters: eyes entire; tempora prominent; antennae filiform, with terminal segment abruptly tapering beyond middle; fourth segment of maxillary palpi excavated laterally on inner side, more than twice as long as third; aedeagus with a pair of large, backwardly directed spines; female genitalia robust, with sides of baculi covered with long spines.

Pergetus Casey differs by the following characters: eyes entire, separated by more than twice their width above; tempora prominent; antennae filiform, with 11th segment abruptly attenuate at middle; fourth segment of maxillary palpi subtriangular, excavated on inner side, as long as the preceding segments combined; pronotum with deep median canaliculation; claws with lobular, membranous empodium; parameres finely and sparsely spined, aedeagus longitudinally serrate at apex; female genitalia robust, with long spines apically (Abdullah 1960).

Mastoremus Casey can easily be differentiated from *Leptoremus* as follows: eyes deeply emarginate and finely granulated; maxillary palpi with last joint cylindrical and only slightly longer than third; vestiture rather sparse, coarse, and not at all concealing the surface sculpture below (Casey 1904).

Bactrocerus LeConte is distinct from *Leptoremus* on the basis of the following features: eyes smaller; surface covered with long, erect hairs which do not conceal the sculpture below; antennae filiform, the last segment as long as all others combined; maxillary palpi with last segment less than twice as long as the penultimate one; labial palpi with terminal segment nearly cylindrical; spines on parameres shorter and less sparse; aedeagus with a pair of large, backwardly directed spines.

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Species Diversity and Insect Population Outbreaks[1]

DAVID PIMENTEL[2]

Cornell University, Ithaca, N. Y.

ABSTRACT

Species diversity and complexity of association among species were found essential to the stability and balance of the community system. The community associated with *Brassica oleracea* L. (Cruciferae) was studied on plants grown in single-species planting and on plants grown in the mixed vegetation of a 15-year fallow field. Approximately 300 species of plants and 3,000 species of heterotrophs existed in the fallow field and these provided species diversity. In 1957, 27 taxa were associated with *B. oleracea* grown in mixed-species planting and 50 taxa were associated with it in the single-species planting. Three to four times as many parasitic and predaceous taxa were present in the single-species planting. Aphid, flea beetle, and lepidopteran populations reached outbreak levels in the single-species planting, but no such outbreaks occurred in the mixed-species planting. These trends were substantiated in 1958. Thus, it was concluded that species diversity play an important role in preventing population outbreaks. Where possible in cultivation, faunal and floral varieties should be encouraged. The use of specific pesticides and biological control agents to remove pests without sterilization should prove a sound practice.

The structure and dynamics of the community system are based on the successful evolution of plants and animals together in a habitat. Diversity of species and complexity of association among species are considered vital to the stability and balance of the community system. Animal population outbreaks occur most frequently in cultivated areas where man has restricted the vegetation to a single species of plant and in the Arctic where simplified communities naturally exist. Ample evidence is available in the literature to support this concept. Marchal (1908) wrote that, "Man in planting over a vast extent of country certain plants to the exclusion of others, offers to the insects which live at the expense of these plants conditions eminently favorable to these excessive multiplication." Later, Graham (1915) wrote, "that pines (white) growing in mixture with other species such as Norway pine (*P. resinosa*) are somewhat less subject to attack" from the white-pine weevil (*Pissodes strobi* Peck). Further support of this observation has come from the works of Peirson (1922), Graham (1926), and MacAloney (1930) who noted that the white-pine weevil was excessively abundant in pure stands of white pine; but when the pine was in with mixed hardwoods, infestations were insignificant. According to von Hassel (1925), insect outbreaks were common in the monocultured forests of Germany but seldom occurred in the virgin forests of South America. Trägårdh (1925), comparing outbreaks in the forests of Germany and Sweden, concluded that fewer outbreaks took place in Sweden because about two-thirds of their forests are mixed pine-spruce-birch. Rohrl (1928) and Friederichs (1928) observed that catastrophes occur in forests where man has changed the environment by monoculture, and they suggested that lack of diversity characteristic of mixed stands was responsible for the outbreaks noted. Damage from two moths was found to be negligible to spruce trees in mixed woods, but in pure stands severe damage often resulted (Barbey 1931). A study of the mango beetle (*Cryptorhynchus gravis* F.) in Java showed that while it causes little damage to the mango fruit when the trees are growing in natural forests, it severely attacks the fruit where the trees are grown by themselves or are otherwise cultured near man's habitation (Voute 1935). In Sumatra, Schneider (1939) reported a lepidopteran (*Oreta carnea Saalm*) in great numbers on cultivated gambir, but in the virgin forests where gambir grows naturally no outbreaks of the pest had ever been observed. Working in Turkey, Schimitschek (1940) reported that the beetle (*Ips sexdentatus* Börn.) is found primarily in pure woods planted by man. Eidmann (1942, 1943) observed that the African virgin forests are relatively free of insect pests in contrast to areas in which man has altered the environment.

The evidence supporting the proposition that more outbreaks occur in single-species stands under man's cultivation than in natural-mixed-species communities, has led some workers to propose that in virgin forests outbreaks never occur (Schimitschek 1937, in Voute 1946). Schneider (1939) wrote further that the virgin forests of the tropics are unsuited for insect plagues because of species diversity. Podhorsky (1933) reported that forests not affected by man seldom suffer from insect pests. In contrast, however, both Schenk (1924) and Eidmann (1942) have pointed out that catastrophes are not restricted to cultivated areas, but that damage occurs occassionally in the virgin forests. They emphasize "occassionally" because, as mentioned earlier, both recognize the fact that outbreaks most frequently take place in cultivated areas.

Population outbreaks occur frequently in the simple natural communities of the Arctic (Elton 1927). Kalabukhov (1947) concludes that population fluctuations of greater amplitude occur in mammal populations in the Arctic because the community contains few species. Further south in the Russian forest-lands and steppes, the animal communities are more complex and these fluctuations are less severe. Support of this analysis is provided by Odum (1953) and Hutchinson (1959).

[1] Accepted for publication June 21, 1960.

[2] Associate Professor of Insect Ecology.

Insecticides, because they are general poisons, tend to sterilize habitat fauna and in so doing reduce diversity. This encourages population outbreaks, as documented in Ripper's (1956) review and the more recent research of Stern and van den Bosch (1959) and Smith and Hagen (1959). This information has lead the University of California ecologists to propose the new concept, "integrated insect control" (Stern et al. 1959).

Thus, considerable evidence in the literature suggests that the lack of species diversity, characteristic of communities modified by cultivation and of natural communities in the Arctic, may be responsible for the population outbreaks which are so typical of these simplified communities. The above information is based on field observation, but in this investigation the relationship between species diversity and population outbreaks was explored by experimentation. Here the quantitative changes in structure of the animal community associated with *Brassica oleracea* L., family Cruciferae, as a single-species stand and then as a member of a more complex plant community were studied and evaluated.

The investigator wishes to acknowledge the conscientious and able assistance of Messers F. A. Streams, E. F. Menhinick, and W. L. Freeman in establishing and maintaining the experimental plots and in aiding in some of the population counting. I wish to especially thank Professors L. C. Cole and B. V. Travis for their helpful suggestions during the preparation of this manuscript.

METHODS

The design of these experiments was influenced by the following hypotheses. First, from evidence available in the literature, more animal species were expected to be present in mixed-species planting than in single-species planting. Second, the density of the specific populations would in general be less in a mixed than in the single-species planting. Third, a greater degree of population stability would be characteristic of the community associated with the plant in a mixed-species planting (complex community) than in the single-species planting (simple community).

The test plots were relatively large, to minimize variability due to either immigrants or emigrants. The plots were separated by distances great enough to reduce variability due to immigration and emigration of animals. Sampling thoroughly and frequently over a long period reduced variability due to changes in plants, temperature, and moisture. Recording the changes found in density in more than one species added support to the general trends. The investigator personally did most of the sampling to reduce variability due to individual differences. Replicating the experiments during two summer seasons reduced the chance of any misleading influence because of weather. The general design, explained in detail below, provided the most satisfactory method for obtaining the information desired using the facilities available to the investigator.

The animal community associated with *B. oleracea* was selected for the study because members of this community are rich in numbers both of species and individuals (Pimentel, unpublished data). In addition, all the animals occur on both natural and cultivated Cruciferae in the Ithaca region. Preliminary observations further showed that the invertebrate herbivores are more attracted to and prefer the cultivated *B. oleracea* species for feeding and oviposition over most wild Cruciferae. Of importance was the fact that the biennial growth characteristics of the plant provides a certain amount of stability to the plant factor in the test environment.

The varieties of *B. oleracea* used in 1957 were cabbage, collards, broccoli, Brussels sprouts, and kale. These differed little in their attractiveness to animals (Pimentel, unpublished data), but collards and broccoli blossomed readily and provided nectar for those individuals (such as adult Lepidoptera and Hymenoptera) that required it, and the broad, flat leaves of collards facilitated insect counting. Hence, collards alone were used in 1958.

At the start of each experiment plants were from 4 to 8 weeks of age. Generally, measurements were made on younger plants in 1958, and this is indicated in the size measurements of the plants.

In the mixed-species planting *B. oleracea* plants were set out in a 15-year fallow field (500 x 600 feet) in which approximately 300 species of plants grew (fig. 1). The mixed-species planting was large so that a greater number of *B. oleracea* plants could be planted than would be possible in a 75 x 100 feet plot. The *B. oleracea* plants were planted in rows at 9-foot intervals both in the row and between rows so as to leave as much as possible of the natural vegetation still growing in the mixed plot. To set out the plants, the soil in a 1-square-foot area was well worked and fertilized and limed. Fertilizer (5-10-5) and lime were applied at a rate of 2,000 pounds per acre to an area of 2 square feet which included the worked area of 1 square foot. A piece of black plastic, 1 foot square, was laid over the worked soil. Through a hole in the center of the plastic, a plant was set in the soil below. The plastic offered the *B. oleracea* plants some protection from competition of the surrounding vegetation.

In addition to the planted *B. oleracea*, five wild species of Cruciferae were present: *Brassica nigra* (L.), *Barbarea vulgaris* R. Br., *Armoracia rusticana* Gaerth., *Cardamine pennsylivanica* Muhl. and *Lepidium campestre* (L.) R. Br. Ample plant food was available for the herbivorous animals associated with *B. oleracea* because most of these species can utilize the wild Cruciferae (Hering 1932). *Barbarea vulgaris* was the most abundant species in the area and from the standpoint of available leaf surface area totaled more than three times that of *B. oleracea* introduced into the mixed-species planting.

The single-species planting was located about 2,000

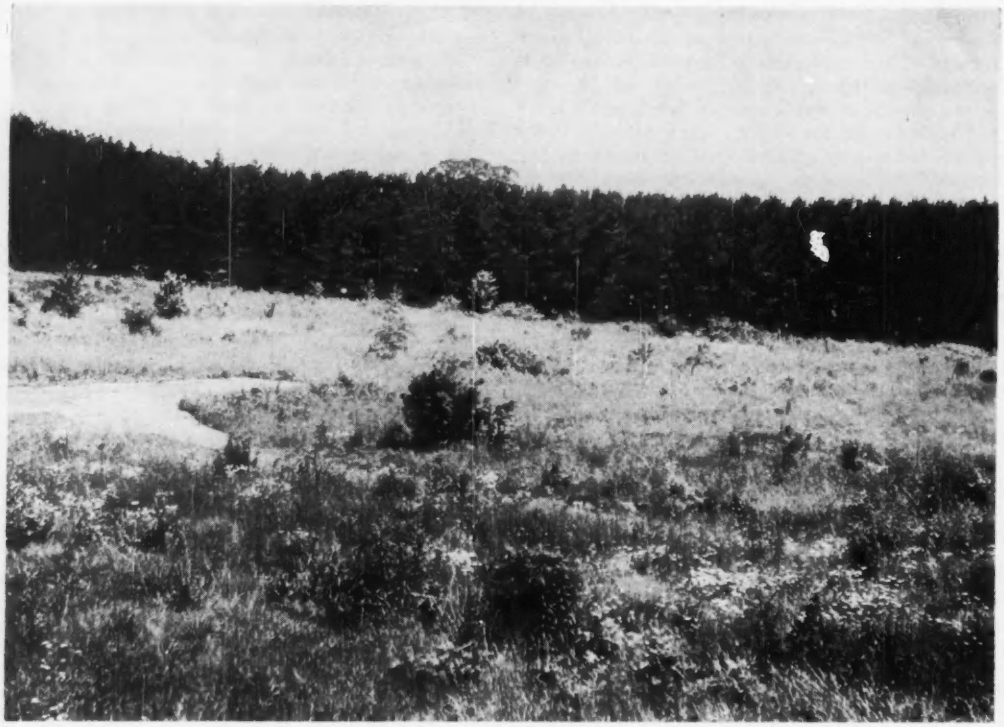

FIG. 1.—A view of the mixed-plant-species habitat.

feet east of the mixed-species planting and measured 75 x 100 feet. This was large enough to prevent any noticeable effect on the populations due to immigrants. In 1957 and 1958 the same varieties of *B. oleracea* were planted there as were planted in the mixed-species planting.

In 1958, another spacing of the single species was added in order to have a plot with plants spaced similarly to those in the mixed-species planting, or 9 x 9 feet from each other. This plot measuring 300 x 900 feet was located about 800 feet east of the single-species planting. Hereafter, this sparse planting of *B. oleracea* will be referred to as the "sparse-single-species planting" in contrast to "single-species planting."

Early in the 1957 season, slugs and field mice destroyed many of the young *B. oleracea* plants. Since this was a behavior phenomenon, not a population phenomenon, and was not present in the single-species planting, 10 percent metaldehyde dust was sprinkled on the plastic below the plants for slugs, and phosphorus-treated corn was distributed to control the mice. These poisons were specific for the slugs and mice. Similar practices were followed in 1958. Any plant destroyed during the experimental period for any reason was immediately replaced with another of similar size obtained from replacements maintained in the greenhouse.

Weekly, 100 plants were measured and animal populations sampled in each plot. Starting the last week in May, the plants were selected systematically to insure a representative sample of the plot. To avoid bias in the choice of individual plants to be sampled, one plant out of every so many, depending upon the total number of plants, was designated for inspection. The outer rows were not included in any sample because of the chance of having edge-effects. An estimate of the total surface area was made by placing a sheet of plastic, which had been etched in 1-inch squares, against the plant and estimating the number of square inches of surface area on the plant. Only one side of a leaf was included in the measurement. The area estimated in this way was demonstrated to have an error of less than 10 percent.

The animal species which make up the community associated with *B. oleracea* are varied, but consist principally of insects. Previous to the initiation of this project, a thorough study was made of the relationship and interactions of the animals of the test community. This information was available to the investigator for reference (Pimentel, unpublished data).

In estimating the number of individuals of any taxon, only a single stage in the life history of each one was used. The stages selected were those which would give the best estimate of the density of the

species and parasitism rates in the species. The following is a list of the types found and the methods by which their densities were determined.

Lepidoptera.—All in the last larval instar were counted. As the larvae were counted, they were collected and placed into separately labeled vials and taken to the laboratory and held to determine rates of parasitism and disease. The pupal stages were not included in the count, but they were collected and held to reveal any parasites which were not common to the larvae.

Homoptera.—All were counted in the adult stage. With the Aphididae, those parasitized and attached to the plant were counted as parasitized. Those individuals from which parasites had emerged were not counted. When more than 100 were present a sample of these was taken; otherwise all parasitized aphids were taken to the laboratory for holding to determine the parasites and their ratios.

Hemiptera.—All were counted in the adult stage. Parasitism was recorded when observed.

Coleoptera.—All were counted in the adult stage. Last-stage larvae of these were collected and held for parasite emergence.

Diptera.—Liriomyza, Scaptomyza, and *Aphiodoletes* groups were counted in the third-larval instar. Pupae were held for parasite emergence. The Syrphidae were counted in the pupal stage and held to determine parasitism rates. All the remaining Diptera types were counted in the adult stage, and the larvae when seen were collected to determine parasitism rates.

Hymenoptera.—All except those species belonging to the family Formicidae were counted as so many parasitic attacks upon their hosts. Their hosts were collected in the field and held for parasite emergence. All other types were counted in the adult stage, and parasitism rates were determined where possible.

Neuroptera.—All were counted in the last larval stage, and they were held in the laboratory to determine parasitism rates.

Arachnida.—All stages were counted and parasitism rates determined where possible.

Other invertebrates.—Any in this category were counted in the adult stage and parasitism was determined where possible.

Microorganisms.—All were counted as so many individual host infections.

In field-sampling the populations, it was difficult to distinguish the differences between some species, genera, and in some cases families. Such organisms were classed into taxa which could be accurately determined in the field. The taxa in all the test communities were handled similarly. A list of the taxa and a discussion of the ecological role of each will be given in a separate paper. The methods of grouping species for counting reduced the categories of taxa in this study to one-fourth of the total noted in the initial survey.

RESULTS

The taxa associated with *B. oleracea* differed in a single-species planting compared to those in a mixed-species planting. In 1957, a total of 27 taxa were found associated with *B. oleracea* grown in the mixed-species planting, 50 taxa in the single-species planting (table 1). In 1958, the differences between the mixed-species and single-species plantings, although significant (39 and 50 taxa, respectively), were not as marked as in the 1957 season (table 1).

Table 1.—The number of taxa which were the same or different in the respective mixed- and single-species stands during the 1957 and 1958 seasons.

Classes	Mixed			Single		
	In 1957 only	Common to both years	In 1958 only	In 1957 only	Common to both years	In 1958 only
Herbivores	4	16	9	1	22	5
Parasites	2	2	4	3	10	1
Predators	0	3	5	5	9	3
Totals	6	21	18	9	41	9

A listing of the taxa observed in each of the experimental plots and the number of individuals counted each week for the 15-week period is filed in the Comstock Memorial Library of Cornell University, and copies of these data may be obtained on interlibrary loan. Although in 1958 the differences between the mixed- and single-species planting were not as great as those of the previous season, they were still significant, 39 and 50 taxa respectively. These taxa were only the ones associated with the *B. oleracea* in the mixed-species planting. Considering the estimated number of 300 plant species in that planting, I would estimate at least 3,000 species of heterotrophs for the mixed-species habitat.

During 1958, the 54 taxa which were reported for the sparse-single-species planting agrees favorably with 50 taxa reported in the single-species planting. Since the spacing of *B. oleracea* plants did not alter the number of taxa appreciably, the differences in number of taxa found in the single and mixed planting cannot be attributed to differences in spacing of the plants.

In the mixed-species planting the taxa number increased from 27 in 1957 to 39 in 1958 (table 1). Of the 27 taxa found in 1957, 21 were common to both seasons. In the single-species planting the number of taxa remained 50 for both seasons (table 1). There was a change in the composition and a total of 41 taxa were common to both seasons. Although a change was made from a few collards plus other varieties of *B. oleracea* planted in 1957 to all collards in 1958, these changes, especially in the mixed-species planting, are not considered responsible for the changes noted in the number and kinds of taxa. Variation in weather is a more valid explanation of the changes. Appreciably lower temperatures in 1958

accompanied by a 42% increase in rainfall would be expected to cause significant changes in the general makeup of the community (table 2).

Table 2.—Mean monthly temperatures and rainfall for the experimental periods in 1957 and 1958.

Year	May	June	July	Aug.	Sept.
	Monthly temperatures (° F.)				
1957	53.9	67.4	67.4	63.6	60.0
1958	52.4	59.2	68.1	66.6	59.2
	Monthly rainfall (inches)				
1957	3.30	3.09	5.55	1.43	3.15
1958	3.37	5.94	5.23	4.83	4.17

Seasonal change in taxa was found to be greater in the mixed planting than in the single planting because the taxa in the mixed planting existed at low densities which made them difficult to measure by the sampling technique. Those taxa which were sparse were the ones most susceptible to variation in detection by sampling. Although a large sample was taken in each plot, not all the plants in any one plot were sampled. A species with few individuals distributed on two or three plants could be missed. Any reduction in number of those taxa which were already marginal for detection would greatly reduce their chances of detection, and, on the contrary, a slight increase in number would increase chances of detection. Thus, because densities of individuals in the mixed planting were marginal for detection by the sampling technique used, a greater change in taxa took place in this plot compared with the single-species planting. This, however, does not alter the fact that there were significantly more taxa per unit plant area in the single compared with the mixed.

In 1957, 20 taxa were common to both the mixed- and single-species planting, and, in 1958, 34 taxa were common to both (table 3). The additional taxa present in the mixed planting were principally herbivores, while in the single-species planting the majority of the additional taxa were parasitic and predaceous. Because 74% of the taxa in the mixed-species planting consisted of herbivores compared with 46% in the single-species planting, a major change in taxa between years in the mixed planting occurred in the herbivore taxa. Many general feeding herbivore taxa in the mixed moved onto the *B. oleracea* plants from the various other plant species in the habitat. However, in the single-species planting herbivores existed at high density and parasites and predators flourished. Thus the greatest change in taxa between years occurred in parasite and predator taxa in the single-species planting.

Table 3.—The number of taxa found only in the mixed- and single- species planting compared with those common to both plantings during 1957 and 1958 seasons.

Classes	Taxa common to both	Mixed only	Single only
	1957		
Herbivores	14	6	7
Parasites	3	1	11
Predators	3	0	12
Totals	20	7	30
	1958		
Herbivores	21	4	6
Parasites	6	0	5
Predators	7	1	5
Totals	34	5	16

The total number of taxa reported for the sparse-single-species planting was 54, which was significantly greater than the 39 reported for the mixed-species planting. A total of 33 taxa were similar in the mixed-species planting and sparse-single-species planting (table 4). The additional taxa found in the mixed were principally herbivores, while most of the other taxa found in the sparse-single-species planting were predominantly parasites and predators. This is similar to findings in mixed-species versus the single-species planting.

Table 4.—The number of taxa found only in the mixed- and sparse-single-species planting compared with those common to both plantings during the 1958 season.

Classes	Taxa common to both	Mixed only	Single only
Herbivores	20	5	9
Parasites	6	0	7
Predators	7	1	5
Totals	33	6	21

Significant changes in the density of the taxa occurred in all the experimental plantings. Variation in plant size and relative abundance of some taxa complicate the presentation of the results. The *B. oleracea* plants in the mixed planting were about one-seventh the size of the plants in the single-species planting at the close of the experimental period in 1957, and about one-fifth in 1958. The mature plants in the sparse-single-species planting were about twice as large as the plants in the single-species planting in 1958. With such differences in size, certain qualitative differences in nutritional content might be expected. No allowances could be made for such qualitative changes, but the quantitative differences in size of plants were considered. To do this the measurements of the plant surface area recorded for each plant were used in adjusting animal densities. All animal densities were made equivalent per unit plant area by adjusting densities to a constant of 20,000 square inches.

Density trends of various groups of taxa are discussed instead of treating each one individually. In 1957 the aphids, especially *Brevicoryne brassicae* (L.), reached outbreak levels in the single-species planting but were scarce in the mixed-species planting (fig. 2). The aphids were not as abundant in 1958 as 1957 in the single-species planting, but they

were still more abundant in the single-species planting than in the mixed-species planting (fig. 3). The difference in aphid density between the single-species and sparse-single-species plots was insignificant.

The other herbivores to reach outbreak levels in the single-species planting were the flea beetles, *Phyllotreta cruciferae* Goeze, *P. vittata* (F.), and *Psylliodes punctulata* Melsh (figs. 4 and 5). The

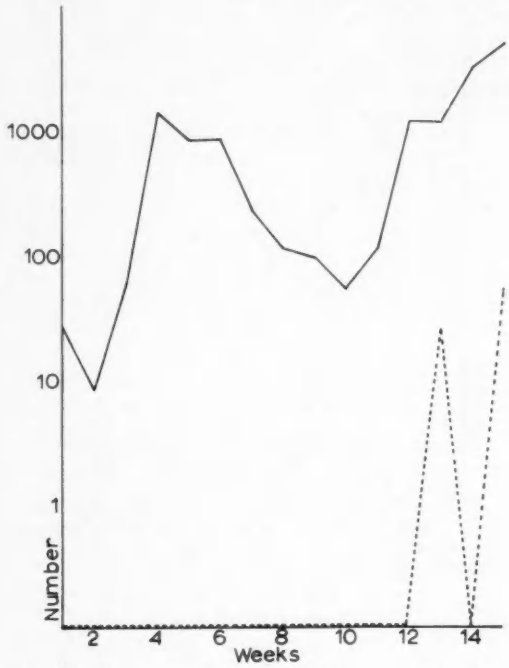

FIG. 2.—The log number of aphids per unit plant area in the mixed-(- - -)and single-species (———) stands during the 1957 season.

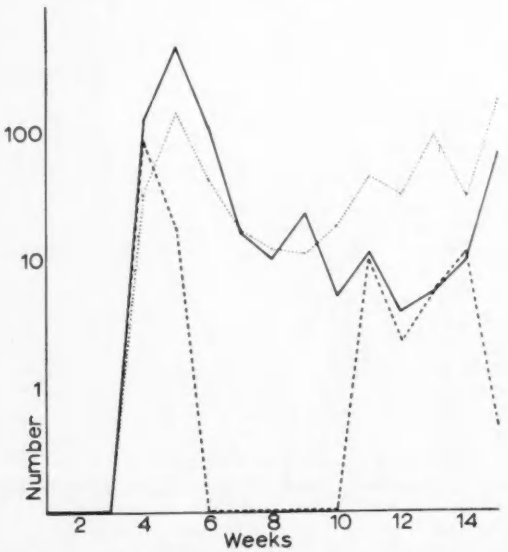

FIG. 3.—The log number of aphids per unit plant area in the mixed- (- - -), single- (———), and sparse-single-species (.....) stands during the 1958 season.

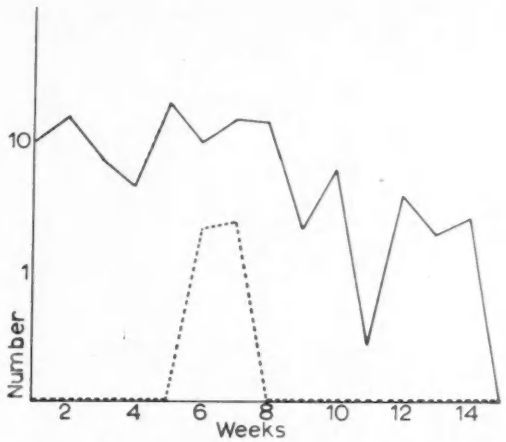

FIG. 4.—The log number of flea beetles per unit plant area in the mixed- (- - -), single- (———), and sparse-single-species (.....) stands during 1957.

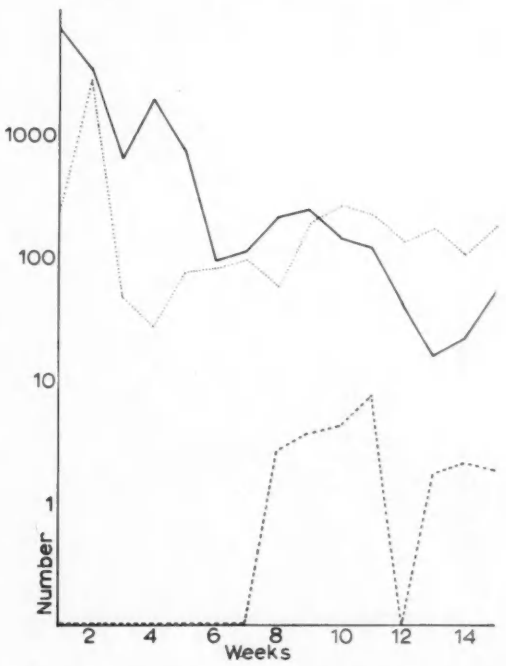

FIG. 5.—The log number of flea beetles per unit plant area in the mixed- (- - -), single- (———), and sparse-single-species (.....) stands during 1958.

flea beetles were most abundant in 1958. The spacing of the plants in the sparse-single-species planting had little affect from the view of diversity on the outbreaks of the flea beetles (fig. 5). As for the Lepidoptera and all other herbivores not mentioned specifically, little seasonal difference was found (figs. 6, 7, 8, 9).

In 1957, the significantly greater number of parasites present in the single-species planting as com-

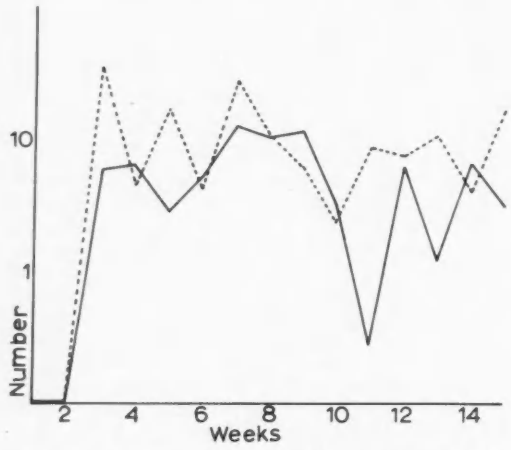

FIG. 6.—The log number of Lepidoptera per unit plant area in the mixed- (- - -) and single-species (———) stands during 1957.

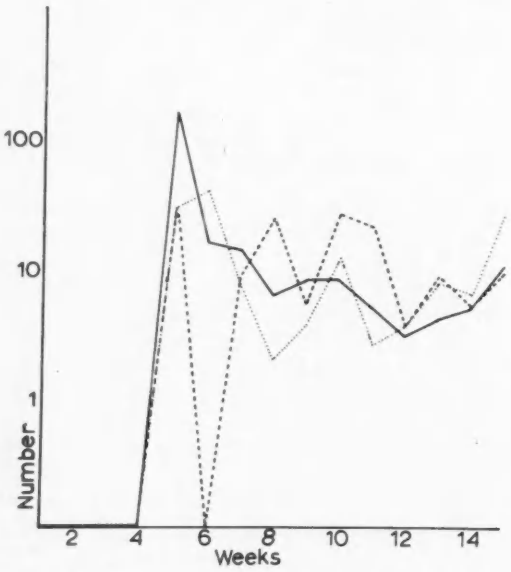

FIG. 7.—The log number of Lepidoptera per unit plant area in the mixed- (- - -), single (———), and sparse-single-species (.....) stands during 1958.

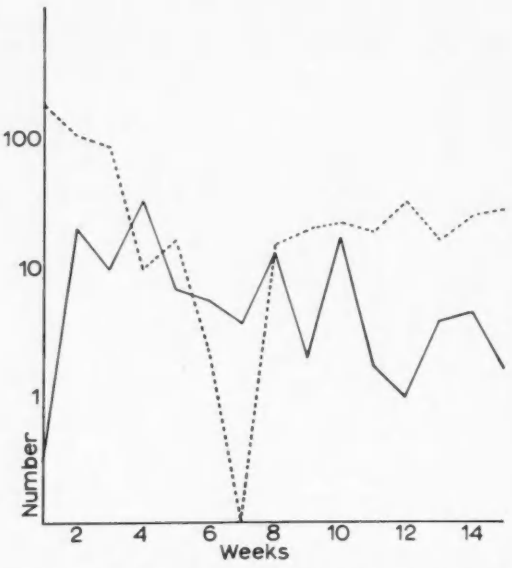

FIG. 8.—The log number of herbivores, other than those graphed in figs. 2, 4, and 6, per unit plant area in the mixed- (- - -) and single-species (———) stands during 1957.

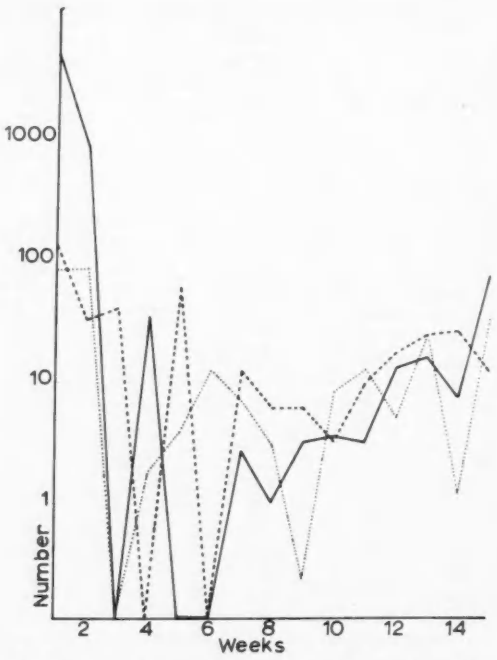

FIG. 9.—The log number of herbivores, other than those graphed in figs. 3, 5 and 7, per unit plant area in the mixed- (- - -), single- (———), and sparse-single-species (.....) stands during 1958.

pared with the number found in the mixed-species planting was directly related to the high *B. brassicae* population in the latter (fig. 10). Because the parasite population had also increased on the aphid population, the ratio of parasites to herbivores was greater in the single-species plot than in the mixed, 18.9% to 8.1%, respectively. These parasite trends were not apparent in 1958, and no difference was found in the number of parasites in either the single-species or mixed-species or sparse-single-species planting (fig. 11). In 1958, however, the parasite-herbivore ratio changed to 12.0% in the mixed, followed by ratios of 4.6% in the single-species, and 4.4% in the sparse-single-species planting. This reversal was mostly due to the change in number of the aphid *B. brassicae* from outbreak level to a markedly lower level in 1958.

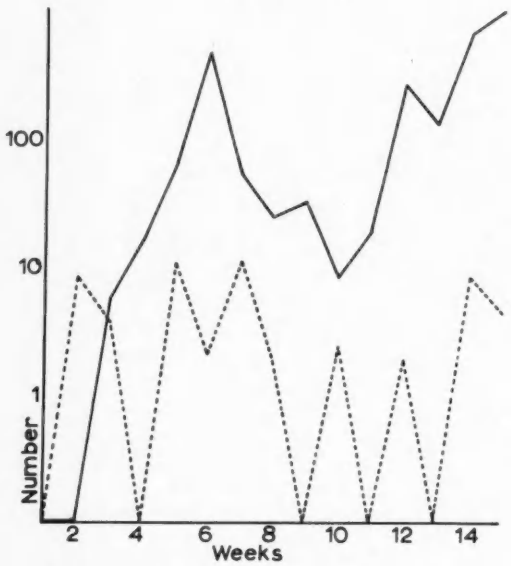

FIG. 10.—The log number of parasites per unit plant area in the mixed- (- - -) and single-species (———) stands during 1957.

Predators which were especially abundant in the single-species plantings in 1957 (fig. 12) were less dense the next season (fig. 13) and paralleled the density of the aphid population. This predator population consisted principally of coccinellids, syrphids, and *Aphidoletes* which were feeding on the tremendous number of aphids present there in 1957. Although in 1957 the total number of predators was greater in the single-species planting, the predator-herbivore ratio was 9.5% in the mixed and 1.1% in the single-species planting. In 1958 a similar trend was evident: a 102.5% ratio in the mixed and 4.5% in the single. As indicated in figure 11, little difference in predator density was noted between the sparse-single

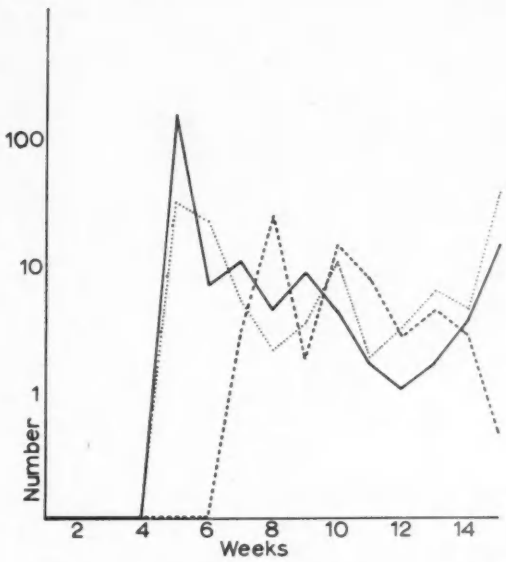

FIG. 11.—The log number of parasites per unit plant area in the mixed- (- - -), single- (———) and sparse-single-species (.....) stands during 1958.

and the other two plantings. The ratio of predators to herbivores in the sparse-single was 6.8%, which is significantly lower than the 102.5% recorded in the mixed. Excluding the single-species planting in 1957, spiders made up the largest proportion of the predaceous fauna in all plantings. One reason for their large number was that all stages were counted, as often nymphal stadia could not be distinguished from adults in the field. However, this trend would still have been evident if only adults had been counted.

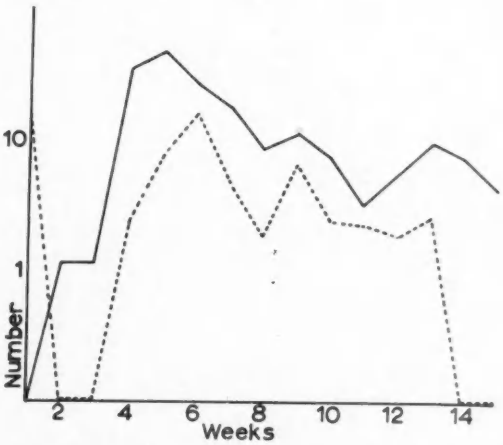

FIG. 12.—The log number of predators per unit plant area in the mixed- (- - -) and single-species (———) stands during 1957.

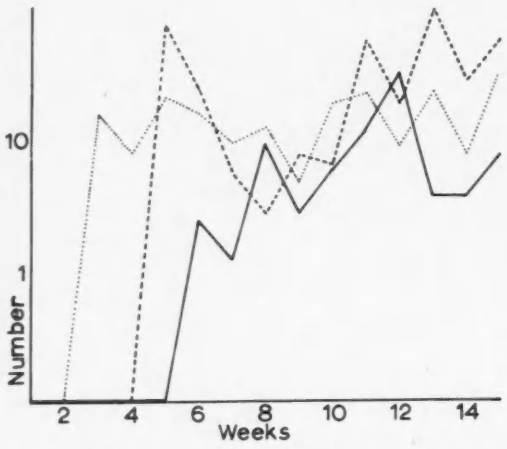

FIG. 13.—The log number of predators per unit plant area in the mixed- (- - -), single- (———), and sparse-single-species (.....) stands during 1958.

DISCUSSION AND CONCLUSION

The significance of this study lies in evaluating the changes in species composition, the density of species, and stability of the community of organisms associated with *Brassica oleracea* when the plant was grown in the single-species stand (simple community) and when grown in mixed-species stand (complex community). These results and the evidence cited in the literature will be considered in the light of some ecological principles.

The ecological principle that species diversity or community complexity functions to stabilize ecologic assemblages, was recognized by workers in the early 1900's. More recently the principle has been restated by Odum (1953), MacArthur (1955), Elton (1958), and Hutchinson (1959). Workers in the field of economics have long relied on the principle of diversity to reduce financial risks. In 1857 the philosopher Herbert Spencer proposed that the trend toward diversity which results in stability has universal application and is the drift of all evolving systems.

Diversity is related in at least three ways to community stability. First, diversity of host and prey species provides alternate food for the parasites and predators and this provides greater stability in these population systems (Nicholson 1933). Each host or prey species reacts differently to the same environmental conditions. One host population may decline as another host population increases. This tends to dampen the oscillations of the interacting host and parasite populations and provides greater stability to the system as a whole. Significantly more host and prey species were present in the mixed-species planting than in the single-species planting, especially when all the host and prey species which were present on the other species of vegetation in the mixed-species planting are included in the total. In this way diversity contributed to the relative population stability observed in the mixed-species planting.

Second, diversity in types of parasitic and predaceous species feeding on one species of herbivore may result in greater stability in these population interactions. With most carnivores there exists a quarry-control range in which the ratio of the number of carnivores to quarry at some quarry density is such that over the range a carnivore species is capable of controlling the quarry population (Holling 1959). At ratios below this range, the quarry population tends to increase until it reaches the effective control range of the carnivore, provided the carnivore population is maintained on supplementary food previous to quarry population increase. If the quarry population can by some chance attain a density greater than the control range, its population then escapes carnivore control. Because the quarry-control range for each species of carnivore is specific for each species, increasing the number of carnivore species generally increases the effectiveness of the control range. Hence, increases in the diversity of species of parasites and predators tend to improve the stability of the natural community. The diversity of parasites and predators in the mixed-species planting probably contributed to the stability of the taxa association with *B. oleracea*.

Third, increased diversity of feeding habits of the species members of a community results in more stability of the organization. Polyphagous predators usually contribute more stability than parasites which are specific in their feeding habits. Predominating in the single-species planting were coccinellids and syrphids which attack mainly aphids. In the mixed-species planting spiders existed, and these are relatively nonspecific in their feeding habits and tend to attack whatever is abundant (Bristowe 1958). Consequently, spiders have more prey species subject to their predation, and this contributed to the greater stability noted in the mixed planting.

The lack of diversity in both the sparse-single-species planting and single-species planting allowed outbreaks to occur. Two other factors may also have been involved in the observed outbreaks. The first concerns a time-lag in the establishment of both carnivores and herbivores in the single-species planting. All taxa had to move into this planting because vegetation was completely destroyed previous to the establishment of the *B. oleracea* plants. In this instance, the investigator doubts that the time-lag factor played a major role in the outbreaks, because wild Cruciferae were flourishing adjacent to all plots and provided ample sources of taxa for invasion of the single-species plots.

The second factor operating in the outbreaks of the single species stand might be termed lack of "community organization." The simple community in the single-species planting is comparable to a subunit of a more complex community unit. Since the simple community is not a functional whole, as the complex community is thought to be, it is con-

sidered less stable. Little is known concerning the exact nature of interspecific integration in communities, but as Emerson (in Allee et al. 1949) has pointed out, it is considered an important factor in community stability. Species members have evolved together to form an integrated and functional system in the community. The parts of this system are balanced as a unit analogous to the functional organization of the parts within an organism (Pimentel 1959). The removal of a subunit will in both instances result in an unbalanced and unstable unit. While this mechanism cannot be singled out as an only cause of the herbivore outbreaks in the simple communities of the single-species planting, it undoubtedly influenced the outbreaks.

The principle that species diversity is important in the control of population outbreaks has application in agricultural crop protection. In fact, the principle has already been applied in silviculture. Graham (1959) recommends diversification as a means of controlling forest pests. Belyea (1923) made a similar recommendation for the control of the white-pine weevil (*Pissodes strobi*) based on his findings that weevil infections were reduced from 290 to 85 per 1,000 trees when the white pines were grown in mixed stands. Although the principle does not have application to all crops, there are some in addition to silviculture where diversification would prove of value for insect control.

In the light of the present findings concerning the value of mixed plantings, the universal recommendation for "clean culture" (Peairs 1947) needs reevaluation. The rule is sound as far as it applies to the removal of crop remains, but the extension of "clean culture" to include the destruction of weeds and hedge rows surrounding the crop area can be seriously questioned. Hedge rows, including weeds and plants of many kinds, may provide diversity with additional species of animals which when added to the community existing in the sterile-crop fields increase its stability. Ullyett (1947) has shown that "where weeds occur around headlands and in hedges, they should be left" for the purpose of supporting parasites and predators important in the natural control of the diamond-back moth (*Plutella maculipennis* Curt.). These hedge rows "form a reservoir for enemies and parasites of insects and mite pests of crops" (Elton 1958).

In conclusion, species diversity is an important factor in preventing population outbreaks in communities. Where possible, diversification of both faunal and floral varieties should be encouraged. The use of specific pesticides and biological control agents which attack only the pest without sterilizing the other fauna should prove to be a sound agricultural practice.

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Larval Nutrition in *Agrotis orthogonia* (Lepidoptera: Phalaenidae): Digestive Enzymes[1]

B. HOCKING AND K. R. DEPNER[2]

Department of Entomology, University of Alberta, Edmonton, Alberta

ABSTRACT

The structure of the alimentary system in the larva of *Agrotis orthogonia* Morr. is described in comparison with published descriptions of related species. A very weak amylase was found in extracts of the ground mandibular glands; a sucrase and a surface active agent were found in extracts of the labial glands; a sucrase, a lipase, and a very weak amylase were found in extracts of the gut. No proteolytic enzymes were found. The results of chemical and microscopical examination of food materials and excreta agreed with these findings. The significance of these results in relation to larval habits is discussed and it is concluded that the insect takes advantage of the breakdown of protein by enzymes in the growing plant.

Since no account of the internal morphology of this insect has been published, the results of investigations into digestive enzymes, our main objective, are preceded by a brief description of the alimentary canal and related structures.

MATERIALS AND METHODS

Larvae were reared from eggs by the method already described (Hocking 1953), using Thatcher wheat seedlings in plaster-bottomed tubes. Batches of newly hatched larvae and of larvae freshly moulted to the second, fourth, and sixth instars were killed and fixed in hot picro-chlor-acetic acid and preserved in 70% alcohol for subsequent sectioning. Incisions were made in the larger larvae before fixing. Larvae were embedded in 65°-71° C. paraffin and serial 10-micron, transverse sections were cut, stained with Delafield's haematoxylin, and mounted in Canada balsam. Sectioning was begun at the posterior end. The morphological information obtained from serial sections was supplemented by dissections of mouthparts from mature larvae and by dissections of third-instar and mature larvae for enzyme studies. Enzyme studies were confined to these two stages.

Extracts in water and in glycerol of the ground-up tissues of washed-out guts and of the mandibular and the labial glands were tested against various substrates. The methods described by Swingle (1928) were used, with the following changes. Chloroform was found to be unnecessary if larvae were chilled; the three regions of the gut were not separated; extracts were tested immediately after preparation. Benedict's reagent replaced that of Fluckiger as a test for reducing sugars. In testing for a lipase, milk was used as a substrate as well as an olive oil emulsion. In the tests for proteolytic enzymes, carmine and congo red were used in place of amaranth and ruthenium red, respectively, and gluten and casein were used as additional substrates. One to four larvae were used in the preparation of each extract from guts and labial glands; six larvae were used for mandibular gland extracts.

In vitro tests of extracts against substrates were supplemented by microscopical examination of faecal pellets and the contents of the different parts of the gut. Chemical tests were made on the food and excreta of larvae fed as described above and of larvae fed on wheat leaf for 3 days previously. Total nitrogen was measured quantitatively by the micro-Kjeldahl method of Kirk (1936) after drying the food and excreta at 100° C., grinding, and redrying. Other tests were qualitative, on aqueous extracts of the dried and ground materials where appropriate.

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[2] Present address: Research Station, Canada Agriculture, Lethbridge, Alberta.

RESULTS

Structure.—Apart from Bordas (1911), workers on the alimentary system of larval Lepidoptera have made exhaustive studies of single species (Lyonet 1762, Peterson 1912) rather than comparative studies of groups. The alimentary system of *Agrotis orthogonia* differs from that of *Prodenia eridania* (Cram.) as described by Woke (1941) in the following respects. The cardiac extension of the mesenteron is strongly divided into two lateral pouches. The pharynx has eight longitudinal ridges instead of six. Four of these, dorsal, ventral, and lateral, are large; four smaller ones, dorsolateral and ventrolateral, lie between them. On these ridges, extending from the middle of the brain forward for about 0.3 mm., are thin-walled hairs called teeth by Woke and, in groups among them, short hairs with large, round knobs on them. Both structures appear to be innervated and are presumably sensory. The mandibular gland is smaller, much smaller than in *Chorizagrotis auxiliaris* Grt. The labial glands are larger, extending back to the seventh abdominal segment, with well-developed accessory glands, and a full S-bend extending through abdominal segments 2 to 4. The spinneret is very short and bilobed; the posterior ends of the glands are tied to the hindgut near the opening of the malpighian tubules by fine threads. The histology of the midgut is similar except that goblet cells are fewer, the peritrophic membrane rather thin, and the striated border of the columnar epithelium appeared less well developed in most of our preparations.

The cryptonephric condition is well exhibited. Nearly 0.5 mm. caudad of the end of the midgut the proximal ends of the malpighian tubules pass inside the muscle layers of the hindgut and run caudad for about 1.5 mm. before opening into the lumen. The openings are funnel-shaped.

The labrum is longer than that of *C. auxiliaris*, with the median notch narrower, deeper, and more heavily sclerotized. The maxillary palpi are also longer. The mandibles are similar, but the adductor muscles are less well developed.

In the first, second, and fourth instars the malpighian tubules do not run backward inside the muscle layers of the hind gut, but open directly into the lumen. The urinary bladders are larger. The relative sizes of the organs and amounts of the tissues differ markedly. Heart and dorsal vessel, musculature, and fat body become progressively larger in the later instars; nervous system and alimentary canal become progressively smaller, in proportion to the body size.

Digestive Enzymes.—Tests on extracts from the gut and from the labial glands of third-instar larvae gave almost identical results to those of mature larvae. These are summarized in table 1. Where negative results are shown, no enzyme was found in at lease six such tests. As a further control, except for cellulase, an extract known to contain the enzyme was tested against the same substrate, using the same reagents. Positive results then were always obtained. Gluten, either unstained or stained with congo red gave very clear-cut results; the surface of the

Table 1.—Results of in vitro tests for enzymes in extracts of guts and mandibular and labial glands of *Agrotis orthogonia* larvae.

Enzyme	Substrate	Test	Alimentary canal		Labial glands		Mandibular glands	
			Test	Control	Test	Control	Test	Control
Water extracts								
Amylase	1% starch	Iodine	±[a]	−	−	−	±	−
		Benedict's	±	−	−	−	±	−
Sucrase	2% sucrose	Benedict's	+	−	+	−	−	−
Lipase	Milk	Phenolphthalein	+	−	−	−	−	−
	Olive oil emulsion	Phenolphthalein	+	−	−	−		
Maltase	2% maltose	Osazone	−	−	−	−	−	−
Cellulase	Filter paper	Benedict's and inspection	−	−	−	−		
Glycerol extracts								
Trypsin	Fibrin	Congo red	−	−	−	−		
	Alkaline casein	Acetic acid	−	−	−	−		
	Gluten	Inspection	−	−	−	−		
Pepsin	Fibrin	Carmine	−	−	−	−		
	Acid casein	Sodium acetate	−	−	−	−		
	Gluten	Inspection	−	−	−	−		
"Erepsin"[b]	2% peptones	Biuret	−	−	−	−		

[a] Some tests failed to give positive results; see text.
[b] An enzyme or enzyme complex capable of splitting peptones into amino acids.

granules became translucent very rapidly in the presence of the active enzyme. No tests were done on larvae which had reached the nonfeeding "prepupal" stage. The tests for amylase were normally incubated for 24 hours; only one weakly positive result was obtained in a test on two mature larvae which was incubated for 48 hours. Food does not remain in the midgut for this length of time.

Measurements of pH, also by the method described by Swingle (1928), gave the following values: foregut 8.4-8.5; mid gut 8.8-9.0.

Table 2.—Results of chemical tests on the materials fed to mature *Agrotis orthogonia* larvae and on the faecal pellets.

	Iodine	Benedict's	Osazone	Biuret	Kjeldahl[a]
Wheat leaf	Very weak	+	+?	+	4.1
Faecal pellets from leaf-fed larvae	Weak	−	−	+	3.1
Wheat seedling	++	++	glucose	+	3.2
Faecal pellets from seedling-fed larvae	++	−	−	+	1.3

[a] Percent total N_2 on dry weight.

When a simulated attack is made on a larva with a pair of forceps, a threatening attitude is adopted with the mandibles and a bubbly glistening white fluid is exuded. The whiteness of this apparently is due to undigested starch grains from the food; it has a pH of 8.4-8.5; shaken up with a little water, it foams readily. The water extracts of the labial glands show similar surface-active properties.

Microscopic examination of gut contents and faecal pellets revealed almost no change in the cellular structure of the plant tissues ingested. In the hindgut and in faecal pellets both lignified and parenchymatous tissues appeared with the cell walls unchanged. Starch grains, too, were found in abundance throughout the alimentary canal and in the faecal pellets. Other contents of the plant cells were progressively reduced in amount through the midgut. The results of chemical tests on the food materials and faecal pellets are summarized in table 2. In table 3 nitrogen assimilation and resultant growth calculated from these results and data from previous work (Hocking 1953) are shown.

While rearing larvae on wheat seedlings it was noted that if there were any differential feeding, the part of the seedling to be consumed first was the coleoptile a few millimetres up from its base. This was investigated further by placing four half-grown larvae in a crock of soil containing wheat plants 2 to 3 inches high. The crock was bare after 9 days; in the later stages the larvae pulled the tops of the plants down into the soil. The maximum rate at which this was seen to happen was 12.5 mm./min. When the remains of the plants were dug out from the soil, all of them were found to consist of an intact root system with seed and a length of coleoptile which varied from 5.9 to 9.6 mm. The actual measurements were 5.9, 5.9, 7.4. 9.2, 9.3, 9.4, 9.6, and 9.6 mm. Three of the larvae were almost mature, the fourth had grown much less.

DISCUSSION AND CONCLUSIONS

Both structural and biochemical evidence indicates that *A. orthogonia* larvae are specialized for feeding on rapidly growing plant tissue. The weak adductor muscles of the mandibles suggest that the fluids in translocation in the plant tissue are of more importance in the diet than the cellular tissues themselves, a suggestion which is supported by the nature of the gut and its contents and by the deficiency of enzymes. That this is necessarily so on account of the high moisture loss resulting from the dry and abrasive environment has already been indicated (Hocking 1953).

Dependence on the fluids in translocation in the plant permits the larva to dispense with some of the enzymes found in related species (Babers and Woke 1937). Or, rather, instead of having to produce these enzymes for itself, it relies on those produced in the tissues of the plant. Digestive enzymes may be even fewer than indicated, since no attempt was made to distinguish intracellular enzymes. The

Table 3.—Mean daily weights of food and faeces, and growth of fifth-instar *Agrotis orthogonia* larvae, all in mg. per specimen.

	Food ingested, dry weight		Faeces passed, dry weight		N_2 assimilated by subtraction	Growth in live weight
	Total	N_2[a]	Total	N_2[a]		
On wheat leaf	25	1.02	20	0.62	0.40	14
On wheat seedlings	34	1.09	27	0.35	0.74	26

[a] By calculation from table 2 and data in Hocking 1953.

apparent inability to make significant use of any nitrogenous material more complex than amino acids, and perhaps the simpler polypeptides, is no handicap to an insect which finds most of the nitrogen in its diet already in this simple form. Since larvae may continue to feed for several hours at one plant, the energy they need for moving from plant to plant is small. This is especially true when they are feeding in field crops where the soil is loose and suitable plants are close together. Presumably this energy is obtainable from sugars and fats, without the need for breaking down starch or cellulose. Possibly some use can be made of starch, but it would be to the advantage of this insect to await the transformation of this material by the plant.

The greater utilization of nitrogen from seedlings than from leaf, as well as the greater amount of moisture available as previously suggested (Hocking 1953), make the former a more satisfactory food. Only 1.6% of the dry weight of leaf ingested is retained nitrogen, as against 2.2% of the dry weight of seedling; or, 68% of the nitrogen in seedlings is assimilated, as compared with 39% of the nitrogen in leaves (tables 2 and 3). This greater utilization may be attributed to the fact that in the rapidly developing seedling a greater proportion of the nitrogen is in relatively simple amino-acid molecules.

Amino acids are most abundantly available, especially in the younger seedlings, in the lower parts of the coleoptile where they are being translocated from reserves in the kernel to the primary meristem (Brown 1946; Fitting et al. 1930). Appropriately, therefore, this is the preferred region for feeding. The exact position at which the plant is cut off appears to depend on the size of the larva. The figures given for the distance above the kernal suggest that the larva holds onto this with the last pair or the last two pairs of thoracic legs and then attacks the coleoptile as far above it as the mandibles can reach. Here the larva intercepts the stream of nutrients, reduced to simple form by plant enzymes, dissolved in water brought by the root system from depths in the soil to which it could not, itself, penetrate.

ACKNOWLEDGMENTS

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COMMON NAMES OF INSECTS

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On the Purported Synonymy of *Piona reighardi* (Wolcott, 1902) with *Piona rotunda* (Kramer, 1879) (Acarina: Pionidae)[1]

ROBERT M. CROWELL

Department of Biology, St. Lawrence University, Canton, New York

ABSTRACT

Life-history studies based on material collected in Ohio indicate that these are two distinct species, so that their recent synonymization by Cook was unwarranted. Eggs laid by *Piona rotunda* in the laboratory yielded nymphs, rather than larvae, upon hatching. Larvae and nymphochrysalids infesting midges, *Tendipes decorus* (Joh.), upon hatching were correlated with *Piona reighardi*, and these nymphs were morphologically distinct from reared and field-collected nymphs of *P. rotunda*. Morphological distinctions in the adults are more evident in males than in females.

In a recent review of the genus *Piona* in the United States, Cook (1960) listed 33 species, including 11 newly described ones. An additional species, *Piona napio* Crowell, 1960, has since been added.

The purpose of the present paper is to dispute the synonymy of *Piona reighardi* (Wolcott, 1902) with *Piona rotunda* (Kramer, 1879), which Cook proposed, and to support the recognition of these as distinct species.

Piona rotunda is of wide occurrence, and has been reported from Europe, Asia, Africa, and North and South America (Viets 1956: 455-6). Koenike (1895: 210) recorded it from Canada; Wolcott (1902: 233) reported its occurrence in Michigan and Nebraska; and Marshall (1935) added collections from Alaska, Montana, and Wisconsin to the previous records.

Piona reighardi, originally described by Wolcott from specimens collected in Michigan, Illinois, and Indiana, has been recorded over a wide area of the United States. To the range just indicated, Marshall (1935: 281-2) added Montana, Iowa, Louisiana, Georgia, and Wisconsin, as well as Ontario. Hoff (1944: 68) reported this species from Tennessee, and Miller (1925: 124) recorded it from Ohio. The writer also has collected specimens of these mites from several localities in Ohio (Crowell 1960). These have been compared with specimens of *P. reighardi* collected by Marshall in Wisconsin, which were loaned by the Chicago Natural History Museum.

It must be agreed that the separation of these species on the basis of adult female morphology is difficult and therefore questionable. In the Ohio material there are slight, but regular, differences in the proportions of the epimeral groups and in the prominence of the anchoral processes on the posterior tip of epimera I (figs. 1 and 9). There is likewise only a slight difference in the palps of the females of these two species—specifically, in the prominence of the seta-bearing papilla on the flexor surface of P-2, the palpal femur (figs. 2 and 10). The palps of *P. reighardi* females are about two-thirds the body length, whereas the palps of *P. rotunda* are less than one-half the body length.

The morphology of the males presents slightly more distinct characteristics which can be used for separation. In males of *P. reighardi* the genital sclerites extend laterally well beyond the projection from the posterior margin of epimera IV, whereas in *P. rotunda* these sclerites extend barely beyond the projection of epimera IV (figs. 3 and 11). The number of genital acetabula is known to vary widely, but in the material studied there are always more than 30 per plate for *P. reighardi*, and under 20 per plate for *P. rotunda*. Among the male specimens studied, the characteristics of the fourth segment of leg IV permit consistent distinction. Leg. IV-4 of *Piona* males shows a prominent medial concavity variously equipped with setae. In *P. reighardi* the proximal edge of this indentation shows three long and four short spines interspersed. The distal edge bears two short spines (fig. 14). In *P. rotunda* there is a row of six stout pegs proximal to the excavation and four distal to it, including one spurlike spine on the distal tip of the podomere (fig. 6). Less pronounced differences may be noted in legs III-6 (figs. 5 and 13).

The development and the morphology of immature stages, however, provide the differences which the writer feels warrant the separation of these species. Females identified as *P. rotunda* produced eggs while kept in laboratory cultures. These eggs, upon hatching, yielded nymphs rather than larvae. Characteristics of one of these are shown in figures 7 and 8. This suppression of the larval stage agrees with the observations of Piersig (cited by Soar and Williamson 1925: 24) for *P. rotunda*.

By repeated collections in the vicinity of Gibraltar Island, Ohio, it has been possible to piece together the developmental cycle of *P. reighardi*. Extensive collections of a midge, *Tendipes* (*T.*) *decorus* (Johannsen), have yielded mite larvae as shown in figure 17, and nymphochrysalids from which nymphs emerged (fig. 15). Morphological similarity of these nymphs to females of *P. reighardi*, and the fact that these were the only pionas found in the area at that time (July 1954), have led to the conclusion that the nymphs, larvae, and adults are of one species.

Comparison of the provisional genitalia of nymphs of the two species reveals marked distinctive characteristics. Nymphs of *P. rotunda* possess two genital acetabula on each side, with a median structure which appears to be an excretory pore (fig. 7). Nymphs of *P. reighardi* (fig. 15) possess five genital acetabula

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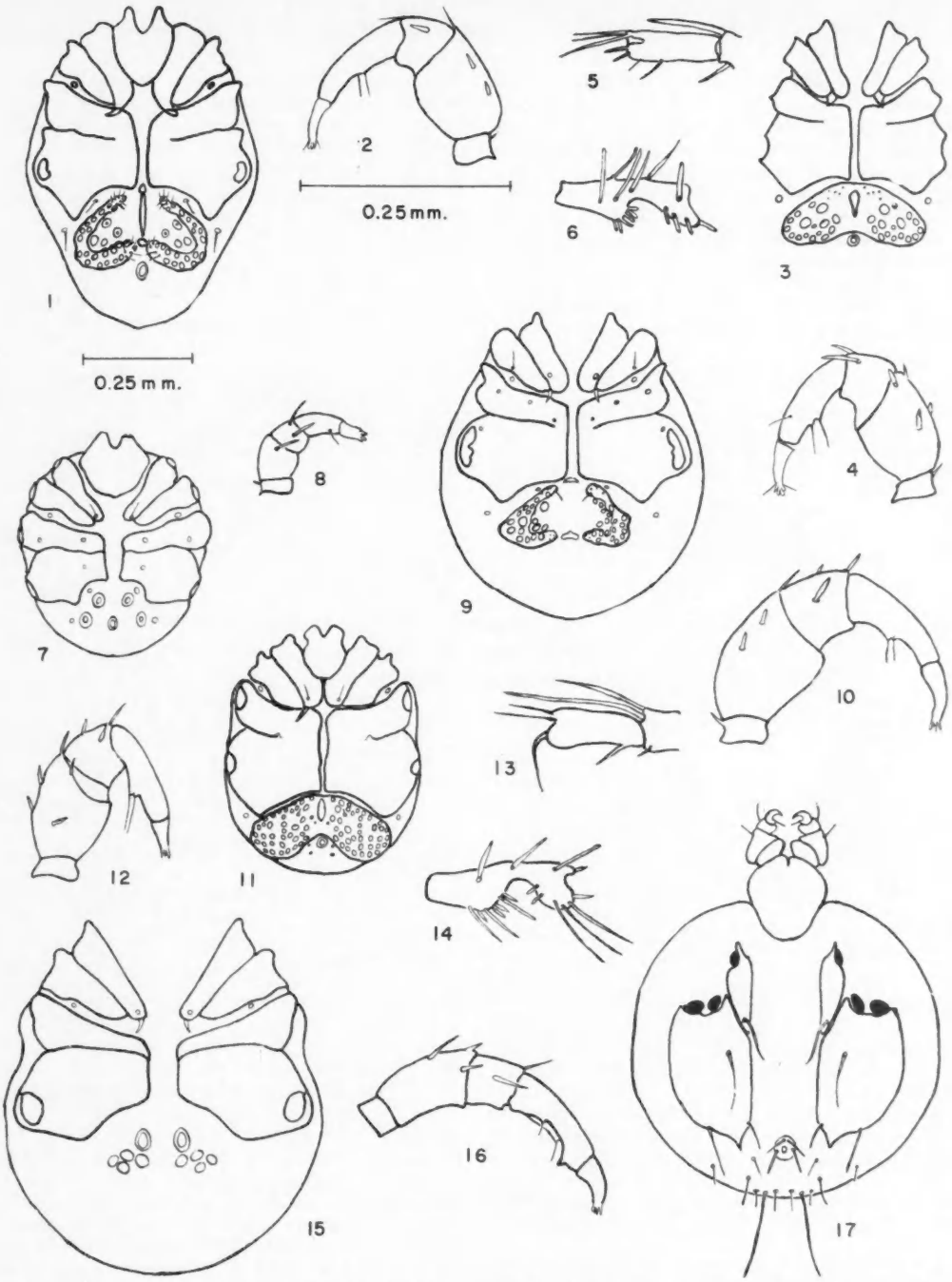

FIGS. 1-8.—*Piona rotunda* (Kramer). FIG. 1.—Female, ventral surface. FIG. 2.—Right palp of female, medial view. FIG. 3.—Male, epimera and genital area; scale as in fig. 1. FIG. 4.—Right palp of male, medial view; scale as in fig. 2. FIG. 5.—Male, left leg III-6; scale as in fig. 2. FIG. 6.—Male, left leg IV-4; scale as in fig. 2. FIG. 7.—Nymph, ventral surface; scale as in fig. 2. FIG. 8.—Left palp of nymph, medial view; scale as in fig. 2.

FIGS. 9-17.—*Piona reighardi* (Wolcott). FIG. 9.—Female, ventral surface; scale as in fig. 1. FIG. 10.—Right palp of female, lateral view; scale as in fig. 2. FIG. 11.—Male, ventral surface; scale as in fig. 1. FIG. 12.—Left palp of male, medial view; scale as in fig. 2. FIG. 13.—Male, left leg III-6; scale as in fig. 2. FIG. 14.—Male, left leg IV-4; scale as in fig. 2. FIG. 15.—Nymph, ventral surface; scale as in fig. 2. FIG. 16.—Left palp of nymph, medial view; scale as in fig. 2. FIG. 17.—Larva, ventral surface; scale as in fig. 2.

on each side, and are much larger than nymphs of *P. rotunda*. Nymphal palps likewise exhibit distinctive characteristics: those of *P. rotunda* (fig. 8) are about half the size of those of *P. reighardi* (fig. 16). Characteristics of palpal segment 4 likewise distinguish the two species.

Because of the suppression of the larval stage in *P. rotunda* and its retention in *P. reighardi*, and because of the evident morphological distinctions in the nymphal stage, this writer feels that these two forms represent valid species and that Cook's relegation of *P. reighardi* to synonymy must be rejected.

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Morphology and Histology of the Alimentary Canal of the Imported Fire Ant Queen (*Solenopsis saevissima richteri*) [1] [2]

JOHN R. WALKER[3] AND DAN F. CLOWER[4]

ABSTRACT

The morphological regions and the histological structures of the alimentary canal of the queen ant, *Solenopsis saevissima richteri* Forel, are described and illustrated. The alimentary canal is composed of the following regions: pharynx, oesophagus, crop, proventriculus, cardiac valve, ventriculus, pylorus, 12 Malpighian tubules, pyloric valve, anterior intestine, rectal valve, and rectum with three rectal pads. The stomodeal region is composed of an internal layer of intima, cuboidal epithelium, longitudinal striated muscle and an external layer of circular striated muscle. In some regions of the stomodaeum the longitudinal muscles are not evident. The mesenteron region is composed of columnar epithelium, circular striated muscle and longitudinal striated muscle. The proctodaeal region is composed mainly of an internal layer of intima, cuboidal epithelium, circular striated muscle and an external layer of longitudinal striated muscle. Strands of longitudinal muscle occasionally are found under the circular muscle.

Literature pertaining to the internal anatomy of the imported fire ant (*Solenopsis saevissima richteri* Forel) is very limited. The morphology and histology of the sting and poison gland of this insect were reported by Callahan et al. (1959).

The results of a study conducted to determine the morphology and histology of the alimentary canal of imported fire ant queens are reported in this paper.

Probably the most comprehensive studies on the internal anatomy of ants were made by Janet (1900, 1902, 1909, and 1923). Many workers consider his studies to be classics in the field of internal anatomy of ants.

In other studies concerning the alimentary canal of ants, Mukerjee (1926) noted that *Dorylus labiatus* did not have a crop and that the proventriculus was poorly developed. Whelden (1957) could not clearly demonstrate the intima or muscle layers in the crop region of *Rhytidoponera*. He noted various amounts of a brown, amorphous material in the alimentary canal, possibly food or waste matter. Forbes (1941) did not find longitudinal muscles in the intestine of *Camponotus*. Eisner (1957) described the various structural diversities in the proventriculus of several species of ants.

MATERIALS AND METHODS

Imported fire ant queens were collected in the Baton Rouge area and the alimentary canals were dissected under physiological saline. The alimentary canals were immediately fixed in Bouin's fluid, washed

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[3] Graduate assistant, Department of Zoology and Entomology, Iowa State University, Ames. Formerly graduate research assistant, Entomology Research Department, Louisiana State University, Baton Rouge.

[4] Associate Professor, Entomology Research Department, Louisiana State University, Baton Rouge.

and dehydrated in ethyl alcohol and cleared in histological xylene. Paraffin was used as an imbedding medium and the sections were cut at 10 microns. The sections were stained with standard alum-hematoxylin and eosin Y. The procedure used was generally that described by Galigher (1934).

RESULTS

Examination of the prepared serial sections reveals that the alimentary canal of the imported fire ant queen is divisible into the following distinct regions.

Pharynx (fig. 1).—The ventral wall of the pharynx consists of a thick, chitinous pharyngeal plate (A). This plate has two arms which extend dorsoposteriorly along the sides of the pharynx. Beneath the pharyngeal plate is a thin layer of epithelium (B). A bundle of muscles (C), arising from the tentorium, is attached to this pharyngeal plate. The dorsal wall of the pharynx is composed of a layer of intima (D) and a layer of epithelium (E). Two bundles of muscle (F), arising from the frons, are attached to this intima. The pharynx becomes progressively less sclerotized dorsoposteriorly and finally is transformed into the oesophagus.

Oesophagus (fig. 2).—The oesophagus has its origin posterior to the insertion of the dialator muscles of the pharynx. It extends ventrad through the brain complex and thorax to terminate at its junction with the crop in the fourth abdominal segment. Internally the oesophagus is lined with a layer of intima (A). The intima is arranged in irregular, convoluted folds from which spines (B) project into the lumen. The epithelium is flattened and evidenced only by scattered nuclei (C). Longitudinal muscles either are not present or are so poorly developed that they are not apparent with the method used to prepare the sections. An outer layer of circular striated muscle (D) surrounds the oesophagus.

Crop (fig. 3).—Histologically, the crop is very

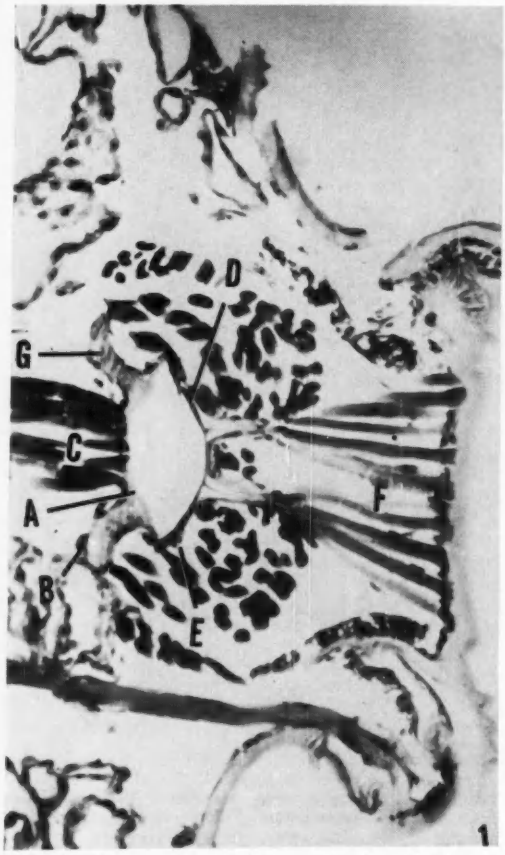

FIG. 1.—Cross section of pharyngeal region: A, pharyngeal plate; B, epithelium; C, muscle attached to tentorium; D, intima; E, epithelium; F, muscles attached to frons; G, pharyngeal plate arms.

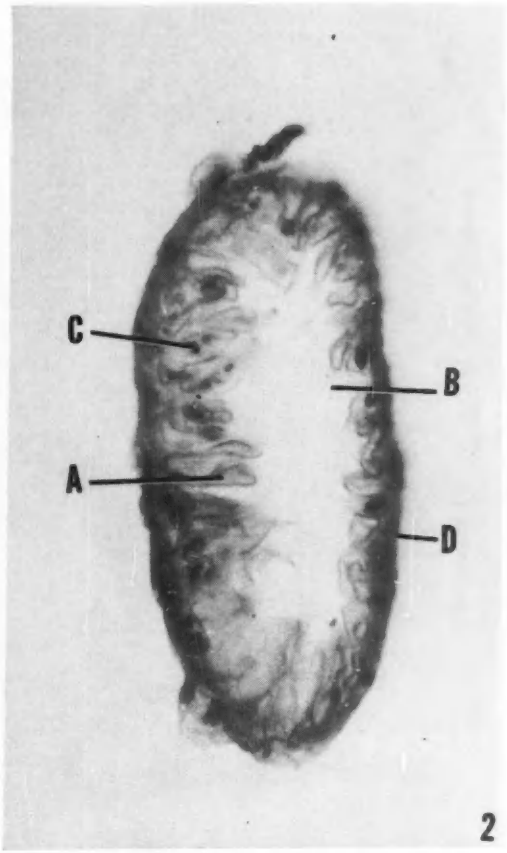

FIG. 2.—Cross section of oesophagus: A, intima; B, spine; C, nuclei; D, circular striated muscle.

similar to the oesophagus. However, the internal layer of intima (A) is thicker than that occurring in the oesophagus. Spines are continuous from the oesophagus and extend into the crop for a short distance. The epithelium is flattened and is very indistinct except for sharply defined nuclei (B). Longitudinal muscles are not evident, but circular striated muscles (C) are loosely arranged around the crop.

The crop was usually found to contain an oily, water-insoluble, yellowish material which presumably was food. This material was also noted by Callahan et al. (1959).

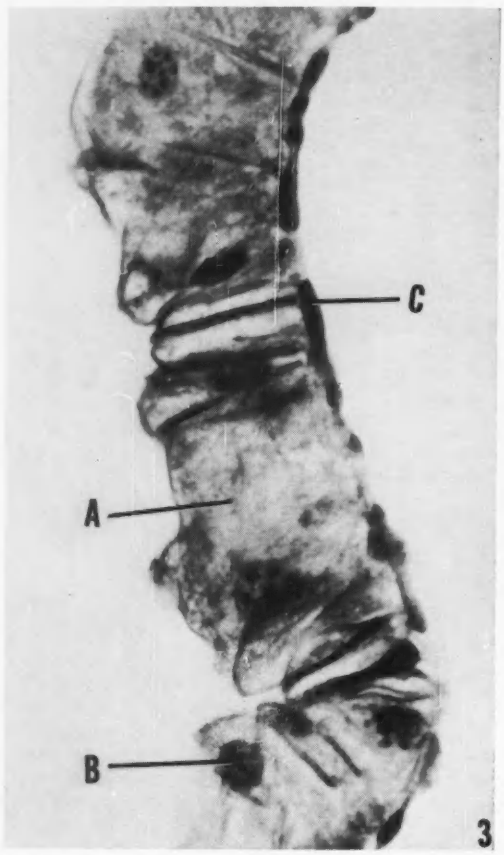

FIG. 3.—Cross section of portion of crop: A, intima; B, nuclei; C, circular striated muscle.

Proventriculus (fig 4).—Externally the proventriculus is composed of from one to three layers of circular striated muscle (A). Beneath the circular muscle there are three or four bundles of longitudinal striated muscle (B) which usually are situated in a fold of the epithelium. The epithelium (C) is arranged in four or five folds and the nuclei (D) are centered on the cuboidal cells. Internally the proventriculus is lined with a layer of intima (E).

Cardiac valve (fig. 5).—A longitudinal section shows that the cardiac valve is a projection of the proventriculus into the lumen of the ventriculus. It is a tube-shaped structure which has invaginated forming a tube within a tube. An intima (A) lines the epithelium (B) of the proventriculus and cardiac valve. At the juncture of the valve with the ventriculus, the epithelium changes from cuboidal to columnar, and the intima terminates. These columnar epithelial cells (C) are smaller than those of the

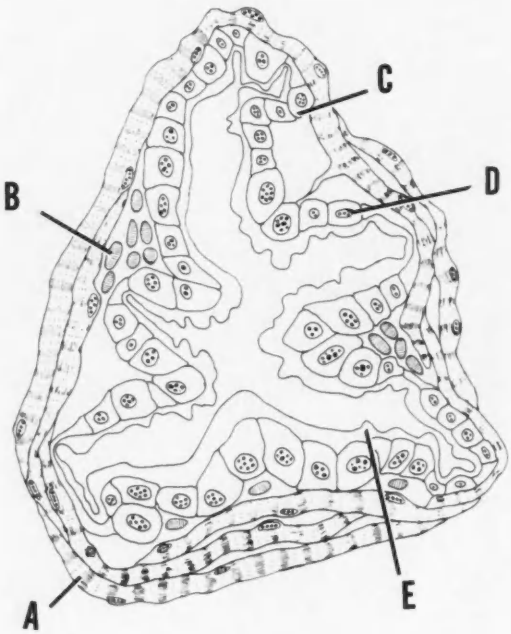

FIG. 4.—Drawing of cross section of proventriculus: A, circular striated muscle; B, longitudinal striated muscle; C, epithelium; D, nuclei; E, intima.

main portion of the ventricular epithelium (D). A layer of longitudinal striated muscle (E) and a layer of circular striated muscle (F) comprise the muscularis of the cardiac valve.

Ventriculus (fig. 6).—The longitudinal striated muscles (A) form a thin, scattered layer over the circular striated muscles (B). There are two types of epithelial cells under this muscularis: large columnar digestive cells (C), and smaller regenerative cells (D). The distal ends of the columnar cells ap-

pear slightly swollen, and there is a necklike constriction just below this enlarged portion on most of the larger cells. The nuclei (E) are located toward the center of the cells. The regenerative cells are pyramidal in shape and are irregularly spaced among the digestive cells. An intima is not present in the ventriculus, but a granular, gelatinous secretion (F) is produced by the epithelial cells which coats their distal ends and the food material as it passes through this region.

FIG. 5.—Longitudinal section of region of cardiac valve: A, intima; B, cuboidal epithelium; C, columnar epithelium; D, ventricular epithelium; E, longitudinal striated muscle; F, circular striated muscle.

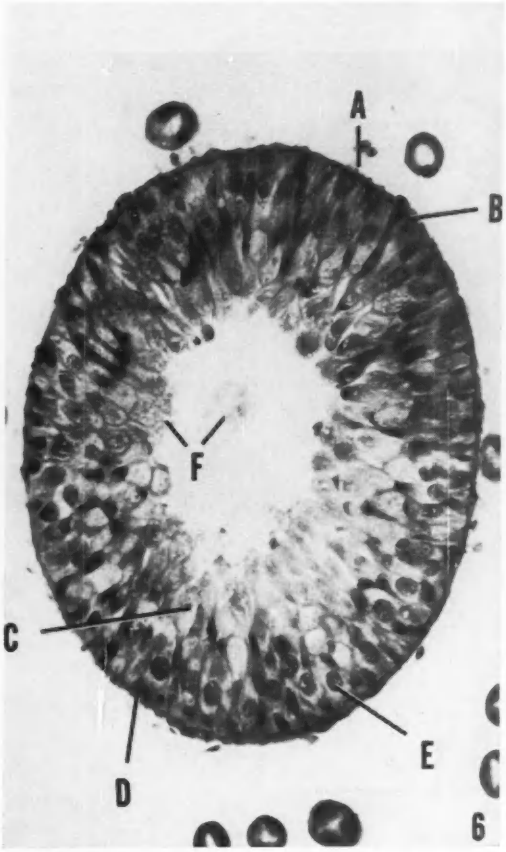

FIG. 6.—Cross section of ventriculus: A, longitudinal striated muscle; B, circular striated muscle; C, columnar digestive epithelium; D, regenerative epithelium; E, nuclei; F, granular, gelatinous secretion.

Pylorus (fig. 7).—The epithelium of the pylorus is flattened and is distinguished only by scattered nuclei (A). An intima lines the epithelium internally. Only occasional strands of external longitudinal striated muscle were found in the sections, but possibly some were destroyed in the preparation of the material. The inner layer of circular striated muscle (C) is evenly distributed over the entire region.

Malpighian tubules (fig. 8).—Radiating from the anterior portion of the pylorus are 12 malpighian tubules. The distal ends of these tubules are free and extend throughout the posterior portion of the perivisceral sinus. When live ants were dissected under physiological saline and the tubules examined immediately, a movement of a brownish granular material was observed within the lumen. However, examination of the serial sections failed to reveal any "cilialike projections." The epithelial cells (A) are cuboidal with the nuclei occupying the center of the cells. Externally, the epithelium is covered by a basement membrane.

Pyloric valve (fig. 7).—The pyloric valve is a ringlike structure (D) which projects into the lumen of the pylorus. It is formed by an enlargement of the epithelium and intima of the pyloric wall immediately posterior to the basal attachment of the malpighian tubules.

Anterior intestine (fig. 9).—An outer layer of

scattered, longitudinal striated muscle (A) encloses the circular striated muscle (B). Occasionally there are strands of the longitudinal muscle found beneath the circular muscle. Inside the layers of muscle there is a single layer of cuboidal epithelium (C). The nuclei (D) of these cells are most frequently located in the basal portion of the cell. The epithelium is arranged in folds and is covered internally by a thin intima (E).

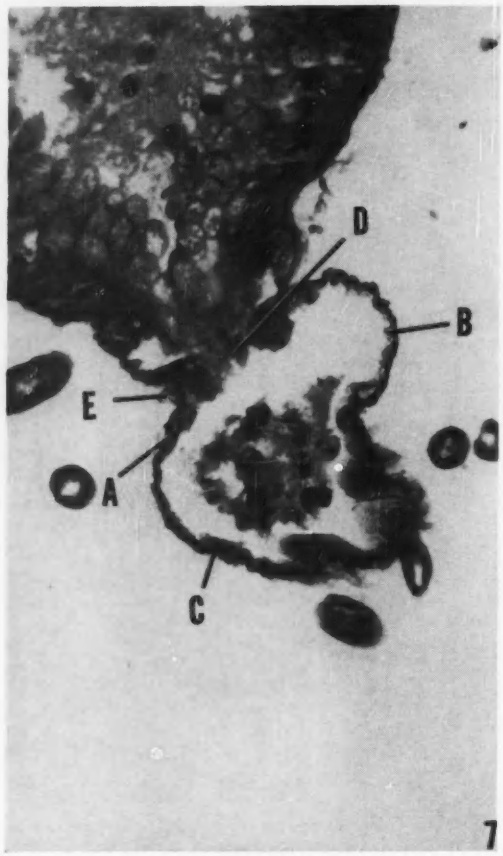

FIG. 7.—Longitudinal section of pyloric region: A, nuclei; B, intima; C, circular striated muscle; D, pyloric valve; E, circular striated muscle.

Rectal valve (fig. 10).—A cross section of the rectal valve region shows the longitudinal and circular striated muscle, epithelium, and intima of the rectum (B). Inside these rectal structures there are two layers of circular striated muscle (A) forming a sphincter around the epithelium and intima of the anterior intestine (C).

Rectum (fig. 11).—The epithelium of the rectum is reduced and represented only by nuclei (A) scattered throughout the intima (B). The intima varies in thickness and is arranged in irregular, convoluted folds. Surrounding the epithelium and intima there is a layer of circular striated muscle (C) and small, scattered longitudinal striated muscle (D). The muscle arrangement in the rectum is irregular. There are occasional strands of longitudinal muscle inside the regular layers of circular muscle.

Rectal pads (fig. 12).—There are three circular-shaped rectal pads located on the rectum. They are

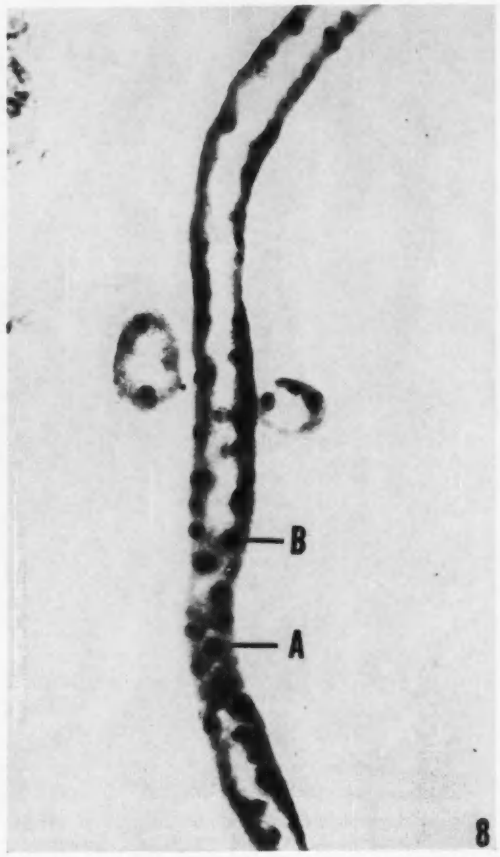

FIG. 8.—Longitudinal and cross sections of malpighian tubules: A, epithelial cells; B, nuclei.

depressed into the wall of the rectum, where they protrude into the lumen. On their outer surface the rectal pads are covered by circular striated muscle and occasional longitudinal striated muscle of the rectum. There are three distinct areas of the pads: basal, central, and distal. The basal area is composed of cuboidal epithelial cells (A) with rounded nuclei (B). A lumen (C) occupies the central area surrounded on either side by a thin layer of cytoplasmic strands with scattered nuclei (D). The cytoplasm of the distal portion of the rectal pads stains deeply

without showing definite cell boundaries. Very granular, deeply staining cytoplasm partially encloses the nuclei (E) in this area. The inner (distal) portion of the rectal pad, which protrudes into the lumen of the rectum, is covered by a thin intima (F).

DISCUSSION

The absence of densely sclerotized areas in the alimentary canal suggested that the queen ant does not feed on solid matter. This hypothesis is further supported by the absence of solid matter in the foregut and the presence of various amounts of a yellow, water-insoluble, oily liquid in the crop. Eisner (1957) stated that adult Hymenoptera subsist largely on fluids which were temporarily retained within the crop.

Both the midgut and the hindgut are found to contain a brownish, amorphous substance. This substance is probably waste matter, since a similar material is observed moving from the distal ends of the malpighian tubules toward the lumen of the alimentary canal. Whelden (1957) noted a similar material, in the same regions of the alimentary canals of *Rhytidoponera convexa*.

The presence of well-developed muscles, from the frons and the tentorium to the pharyngeal region, suggest that the pharynx is a region adapted as a sucking organ. These muscles, when contracted, would expand the pharyngeal lumen and exert a slight negative pressure, possibly drawing food from the buccal cavity into the pharynx. The absence of both circular and longitudinal muscles around the pharynx suggested that if it does function as a pumping organ it is the elasticity of this organ which acts as antagonist to the dilator muscles.

The arrangement of the tissues composing the regions of the alimentary canal is summarized in table 1. The regions of the alimentary canal are listed in order from the anterior to posterior end of the body. The arrangement of the composing tissues are listed in order from the innermost to the outermost layers.

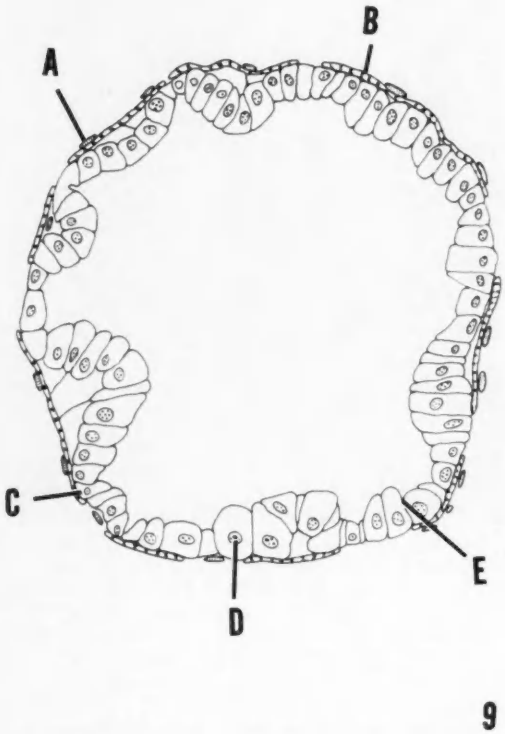

FIG. 9.—Drawing of cross section of anterior intestine: A, longitudinal striated muscle; B, circular striated muscle; C, cuboidal epithelium; D, nuclei; E, intima.

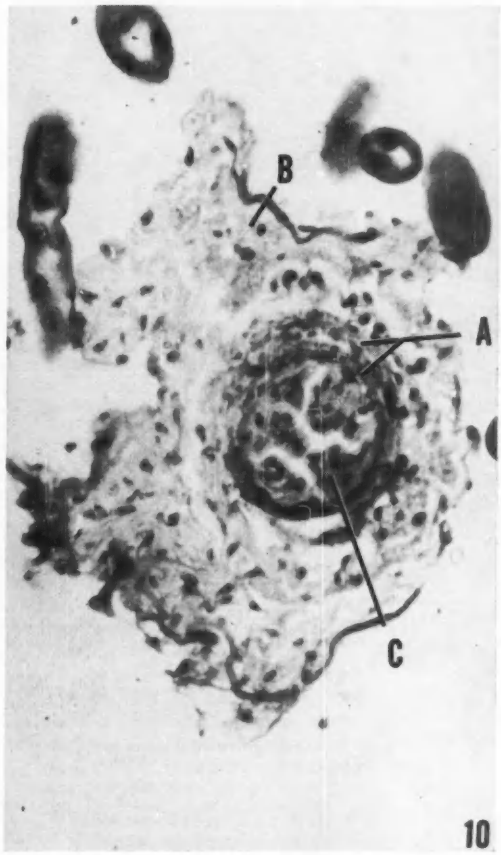

FIG. 10.—Cross section of rectal valve: A, circular striated muscle; B, rectum; C, anterior intestine.

FIG. 11.—Oblique section through rectum: A, nuclei; B, intima; C, circular striated muscle; D, longitudinal striated muscle.

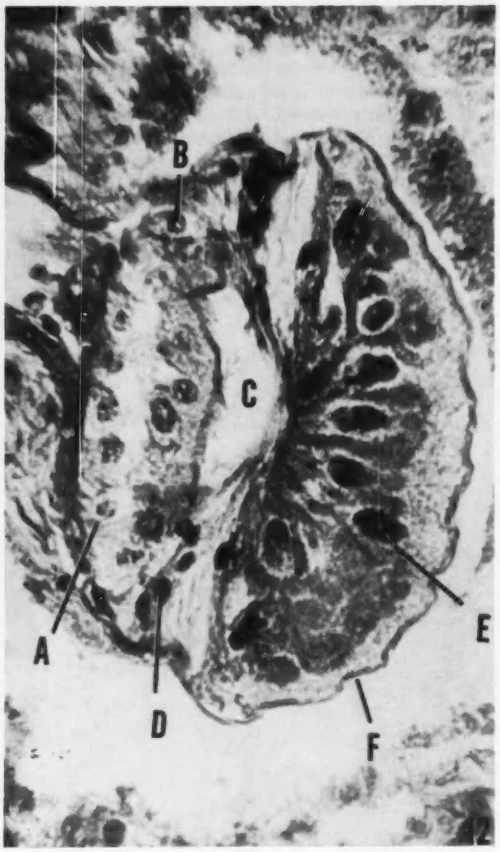

FIG. 12.—Cross section of rectal pad: A, cuboidal epithelium cell; B, nuclei of basal area; C, lumen; D, nuclei of central area; E, nuclei of distal area; F, intima.

Table 1.—The regions of the alimentary canal and the composing tissues.

Region of the alimentary canal (anterior to posterior)	Arrangement of the composing tissues (innermost to outermost)
Pharynx	chitinous intima, flattened cuboidal epithelium, striated muscle attached to the frons and the tentorium.
Oesophagus	chitinous intima with spines, flattened cuboidal epithelium, circular striated muscle.
Crop	chitinous intima with some spines, flattened cuboidal epithelium, circular striated muscle.
Proventriculus	chitinous intima, cuboidal epithelium, longitudinal striated muscle, circular striated muscle.
Cardiac valve	chitinous intima, cuboidal epithelium changing to columnar epithelium, longitudinal striated muscle, circular striated muscle.
Ventriculus	peritrophic membrane, columnar digestive and regenerative epithelium, circular striated muscle, longitudinal striated muscle.
Pylorus	chitinous intima, flattened cuboidal epithelium, circular striated muscle, occasional longitudinal striated muscle.
Malpighian tubes	cuboidal epithelium, basement membrane.
Pyloric valve	chitinous intima, enlarged cuboidal epithelium, sparse circular striated muscle.
Anterior intestine	chitinous intima, cuboidal epithelium, circular striated muscle, longitudinal striated muscle.
Rectal valve	anterior intestine, 2 layers of circular striated muscle, rectum.
Rectum	chitinous intima, flattened cuboidal epithelium, circular striated muscle, longitudinal striated muscle (occasionally some inside circular muscle).
Rectal pads	chitinous intima, granular cytoplasm, lumen, cuboidal epithelium, covered by rectal muscles.

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Lipogenesis from C^{14}-Acetate by the American Cockroach[1]

SPIRO J. LOULOUDES, J. N. KAPLANIS, W. E. ROBBINS, AND R. E. MONROE

Entomology Research Division, Agr. Res. Service., U.S.D.A., Beltsville, Md.

ABSTRACT

Adult American cockroaches (*Periplaneta americana* L.) were injected with an aqueous solution of 1-C^{14}-sodium acetate and held for 24 hours. On analysis, the rate of fatty acid synthesis from C^{14}-acetate was found to be 2.5 to 4.7 times greater in male than in female cockroaches. Analysis of the fatty acid methyl esters by gas-liquid chromatography demonstrated the relative distribution of radioactivity to be similar for both sexes. Both the male and female cockroaches incorporated approximately the same percentage of radioactivity into the unsaponifiable fraction. Fractionation by column chromatography demonstrated that 59% to 68% of the radioactivity behaved as hydrocarbons and 5% to 8% as sterols. None of the radioactivity in the hydrocarbon fraction behaved as squalene when analyzed by paper chromatography. Only low levels of radioactivity were found in the digitonin precipitates of the sterol fraction, none of which showed distinct peaks corresponding to either the Δ^5 or $\Delta^{5,7}$ sterol acetates following acetylation and chromatography on alumina.

The two-carbon moiety, acetate, is an important precursor to a wide variety of lipids in vertebrates. In insects the larvae of the hide beetle, *Dermestes vulpinus* Fabr. (Bloch et al. 1956), and the adult house fly, *Musca domestica* L. (Robbins et al 1960), use C^{14}-acetate for the biosynthesis of both saponifiable and unsaponifiable lipids. The adult American cockroach, *Periplaneta americana* L., has been reported to incorporate C^{14}-acetate into the unsaponifiable fraction, a significant portion of which was found to be digitonin-precipitable material (Casida et al. 1957). This study was initiated to evaluate the role of acetate in fatty acid synthesis and to investigate the nature of the radioactive compounds in the unsaponifiable fraction of the adult American cockroach.

MATERIALS AND METHODS

Adult American cockroaches reared and maintained on Purina dog chow were used in these experiments. Groups of 20 cockroaches of each sex were injected in the abdomen with an aqueous solution of 1-C^{14}-acetate (table 1). After 24 hours the insects were sacrificed and held frozen until analyzed.

The cockroaches were homogenized in a Waring blendor and saponified under nitrogen with alcoholic potassium hydroxide. The unsaponifiable and saponifiable lipids were extracted with diethyl ether, and the ether extracts washed with water, dried over anhydrous sodium sulfate, and concentrated in vacuo. The fractions were then adjusted to known volumes and aliquots plated for radioassay.

The unsaponifiable lipids were pooled according to sex, and a portion analyzed for sterols by digitonin precipitation, as described by Casida et al. (1957). The digitonides, following radioassay, were then cleaved with pyridine and the free sterols acetylated and cochromatographed on alumina (Kaplanis et al. 1960). The remainder of the unsaponifiable material was subjected to column chromatography on Woelm acid aluminum oxide (Robbins et al 1961), which separated hydrocarbons and sterols.

The hydrocarbon fraction was further analyzed by paper chromatography using a modification of the method of Dauben et al. (1952). One-inch strips of Whatman No. 1 paper impregnated with a 2.5% Quilon solution served as the stationary phase. The chromatograms (20-cm. runs) were developed by the ascending method in glass-stoppered graduate cylinders containing 50 ml. of the mobile phase, methanol-isooctane 9:1 (v/v).

The sterol fraction from the column, eluted with ether, was analyzed by digitonin precipitation. Ali-

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Table 1.—Summary of treatment data.

	Males[a]	Females[a]
Av. weight/roach (gm.)	0.93	1.23
μgm. C^{14}-acetate[b] roach	100.	130.
Total cpm ($\times 10^8$)	2.58	3.36

[a] Two groups of 20 roaches.
[b] Specific activity 6.46×10^4 cpm per μgm.

quots of the ether eluate were transferred to centrifuge tubes, the ether evaporated, and the residue dissolved in acetone-absolute ethanol 1:1. The digitonin was added as a 1% solution in 85% ethanol, and the digitonides were allowed to precipitate (Sobel and Mayer 1945). After successive rinsing with acetone-absolute ethanol, acetone-ether, and ether, the digitonides were weighed and then cleaved with pyridine (Schonheimer and Dam 1933). The liberated sterols, recovered by extraction with anhydrous ether, were subjected to a second digitonin precipitation after radioassay. Samples were prepared for analysis by plating 1 to 10 mg. of the sterols from a benzene solution (1 mg/50 μl.) to planchets lined with filter-paper discs.

The saponifiable fraction from the roaches was analyzed by gas-liquid chromatography. The fatty acids were esterified with diazomethane (DeBoer and Backer 1954), and aliquots of the methyl esters were then chromatographed at 214° C. on a 10 foot by 1/4 inch stainless steel column packed with siliconized chromasorb W (60-100 mesh) coated with 16.2% adipate polyester (Lac 2-R 446) as the polar liquid phase and helium (76 ml./min.) as the carrier gas (Horning et al. 1959). A thermal-conductivity cell was used as the detector, and the fatty acids were identified by both their chromatographic pattern and retention volumes. Fractions were collected for radioassay in centrifuge tubes containing *n*-hexane immersed in a dry ice-acetone bath. Samples were prepared by plating aliquots of the fractions into planchets coated with cholesteryl acetate. The sterol ester reduced the vapor pressure of the C^{14}-fatty acid esters and prevented contamination of the counting chamber.

Radioassays were made with a gas-flow proportional counter. All samples were prepared in duplicate or triplicate, usually as weightless samples, and counted for a sufficient time to give a standard error of ±5%. Where applicable, the counting rates were corrected for self-absorption.

RESULTS

The incorporation of radioactivity into the lipid fractions by the adult American roach is given in table 2. Both sexes utilized considerably more of the injected acetate for the synthesis of the saponifiable than for the unsaponfiable lipids. The amount of C^{14} compounds in the saponifiable fraction was several times greater in males than in females, whereas the rate of incorporation into the unsaponifiable fraction was found to be similar for the two sexes.

Table 2.—Incorporation of 1-C^{14}-acetate into the saponifiable and unsaponifiable lipids of adult American cockroaches 24 hours after treatment.

Group	Total cpm ($\times 10^5$)	Weight (mg.)	Percent of total dose
	Saponifiable		
Male			
1	238.8	977.6	18.5
2	218.1	846.9	16.9
Females			
1	52.9	879.1	6.9
2	29.9	942.3	3.9
	Unsaponifiable		
Male			
1	1.45	159.6	1.1
2	1.57	144.5	1.2
Females			
1	7.12	197.9	0.9
2	6.92	177.6	0.9

When the unsaponifiable fraction was analyzed by column chromatography (table 3), more than 59% behaved as hydrocarbon(s) and only 5% to 8% as sterols. Further analysis of the hydrocarbon fraction by paper chromatography demonstrated the presence of two radioactive peaks. Neither the major (R_f 0.31) nor the minor radioactive compound(s) (R_f 0.13) behaved like squalene (R_f 0.47) when cochromatographed with an authentic standard.

Table 3.—Column chromatography of radioactive unsaponifiable lipids from adult American cockroaches.

Fraction	Females[a]		Males[a]	
	Total cpm ($\times 10^4$)	Percent of unsaponifiable	Total cpm ($\times 10^4$)	Percent of unsaponifiable
Hexane (hydrocarbons)	47.1	68.7	85.6	59.0
Benzene	2.0	2.9	4.4	3.0
Diethyl ether (sterols)	5.4	7.9	7.2	5.0
Methanol	1.1	1.6	2.5	1.8
Per cent Recovery		81.1		68.8

[a] Average for two groups of 20 roaches.

The data in table 4 indicate a higher incorporation of radioactivity in sterol digitonides of the female roach than in the male when either the total unsaponifiable lipids or the sterol fraction from the column was subjected to digitonin precipitation. Direct precipitation of the unsaponifiable fraction gave higher counting digitonides with both sexes, and a marked decrease of radioactivity in the reprecipitated digitonides from the males. When the free sterols from the digitonides were acetylated and cochromatographed on alumina with standards, no definite radioactive peak was found in either the Δ^5 or the $\Delta^{5,7}$ sterol acetate fraction.

Gas-liquid chromatograms of methyl esters prepared from the saponifiable fractions demonstrated the presence of 17 fatty acids (fig. 1, curve A), 9 of which were identified by comparing their chromatographic patterns and retention volumes with those of

Table 4.—Digitonin precipitation of radioactive compounds from the unsaponifiable fraction of American cockroaches injected with 1-C^{14}-acetate.

Digitonin precipitation	Sterol fraction from unsaponifiable lipids		Total saponifiable lipids	
	Male	Female	Male	Female
First precipitate				
(Total cpm)	293	8108	1845	13,085
Incorporation (per cent)	2.3×10^{-4}	1.1×10^{-2}	1.4×10^{-3}	1.7×10^{-2}
Second precipitate				
(Total cpm)	53	7947	118	[a]
Incorporation (per cent)	4.1×10^{-5}	1.0×10^{-2}	9.1×10^{-5}	

[a] Acetylated and analyzed by column chromatography on alumina.

authentic standards (fig. 1, curve B). The remainder were tentatively identified by plotting the relative retention times vs. number of carbon atoms in the fatty acids (fig. 2).

The distribution of radioactivity in the fatty acids was similar for both sexes (table 5). Oleate showed

Table 5.—Relative distribution of radioactivity in fatty acids from American cockroaches injected with 1-C^{14} acetate.

Fraction No.[a]	Males		Females	
	Total cpm	Per-cent	Total cpm	Per-cent
1 Caprylate and hendecanoate	26	0.2	Trace	<0.1
2 Myristate	62	0.4	55	0.8
3 Palmitate and palmitoleate	4699	30.7	1838	26.8
4 Margarate	76	0.5	21	0.3
5 Stearate	1522	9.9	685	10.0
6 Oleate	6745	44.1	3150	45.9
7 Linoleate	1509	9.9	788	11.5
8 Linolenate	662	4.3	316	4.6

[a] Only the major fatty acid(s) are included here. Figure 1, curve A, shows the fatty acids present in each fraction.

the greatest incorporation, accounting for about 45% of the radioactivity. Another 27%-31% was found in the palmitate-palmitoleate fraction, and stearate and linoleate each contained about 10% of the radioactivity.

DISCUSSION

The American cockroach incorporates C^{14}-acetate into the unsaponifiable lipids at approximately the same rate found for the house fly (Robbins et al. 1960). The unsaponifiable fraction in both of these insects appears to be a very dynamic system when compared with this fraction from the rat (Hutchens et al. 1954). As in *Dermestes* larva (Clark and Bloch 1959) and the house fly (Robbins et al. 1960), most of the radioactivity in the unsaponifiable material of the roach was found in the hydrocarbon fraction. Clark and Bloch (1959) present evidence that the C^{14}-hydrocarbons from the former insect are high-molecular-weight straight-chain compound(s). However, both the house fly and cockroach hydrocarbon(s) have been found to be complex mixtures, including branched chain and/or unsaturated compounds, when analyzed by gas chromatography and mass spectrometry (unpublished data). Additional work on the characterization of these compounds is in progress. The absence of a biochemical mechanism for squalene synthesis in the American cockroach is in agreement with the findings for both *Dermestes* larvae (Clark and Bloch 1959) and adult house flies (unpublished data).

Only low levels of radioactivity were detected in the sterol digitonides. The marked decrease in activity after reprecipitation of the digitonides from the total unsaponifiable lipids suggests occlusion of other labeled substances, possibly hydrocarbons, which are insoluble in the solvents used for digitonide formation. Small amounts of radioactivity in the sterol digitonides from the female roach may be due to the synthetic activity of symbiotic and/or intestinal microorganisms or may represent nonsteroidal compounds which form digitonin precipitates (Fieser and Fieser 1949). Chromatographic analyses of the acetylated sterols from the digitonides failed to show definite radiometric peaks corresponding to either Δ^5 or $\Delta^{5,7}$ sterol acetates, indicating that these radioactive compounds are probably not sterols. Thus, the absence of any significant incorporation of acetate into sterols by the American cockroach is in keeping with the prior nutritional and biochemical data for insects.

The radioactivity in the fatty acids of the male roach was 14 to 17 times greater than for the unsaponifiable fraction, whereas in the male house fly these fractions are nearly equal. Notable sexual differences between the two species are apparent when the saponifiable fractions are compared. Unlike the male house fly, the adult male cockroach utilized considerably more acetate for fatty acid synthesis than the female cockroach. This reversal of a sexual difference in lipid synthesis and turnover between the two species of insects merits additional study.

The high rate of incorporation of acetate into the saponifiable lipids and the large number of fatty acids found to contain the labeled moiety point to the American cockroach as a promising insect for the study of fatty acid synthesis. The relative distribution of the radioactivity found in the fatty acids

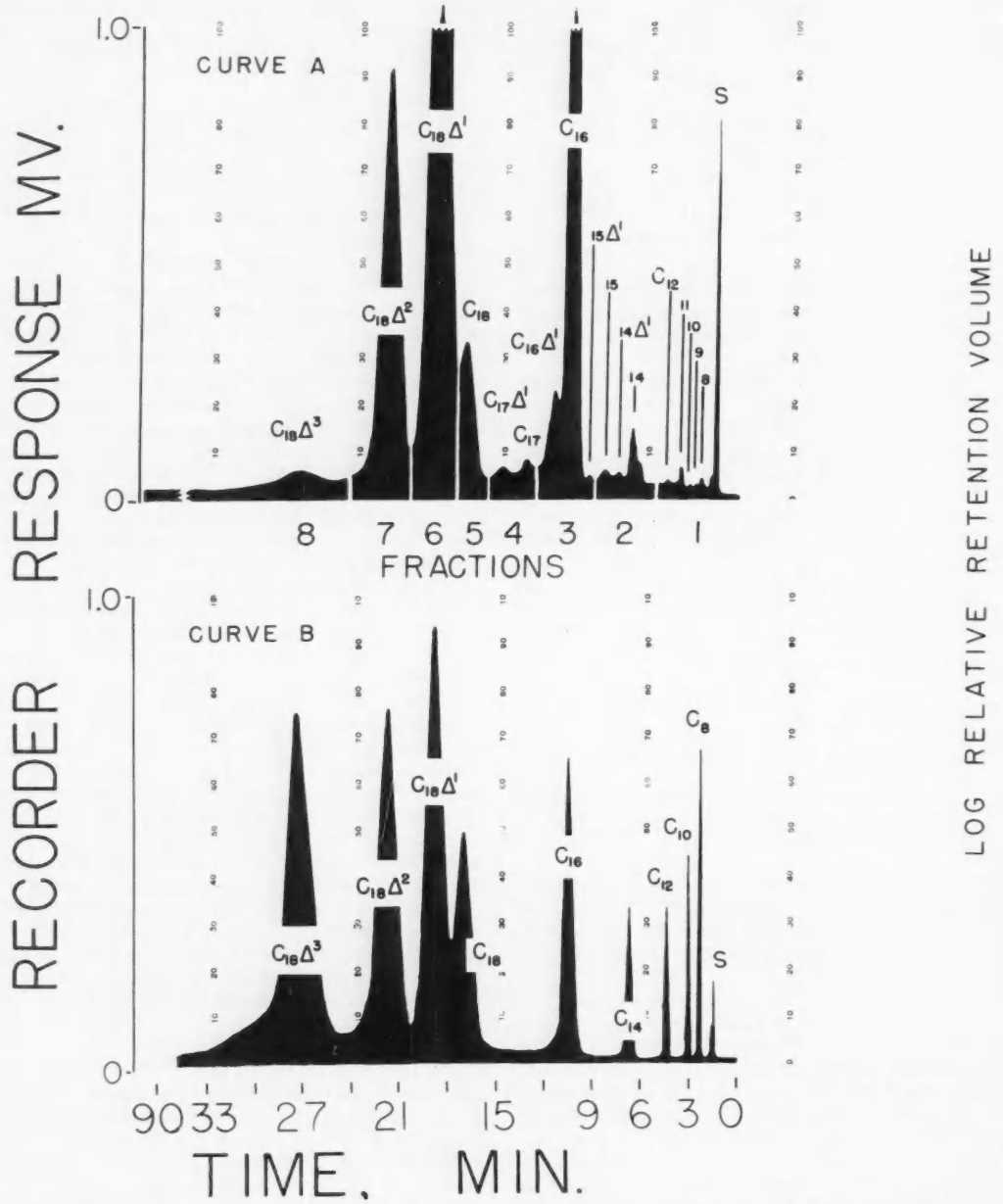

FIG. 1.—Separation of methyl esters of fatty acids by gas-liquid chromatography. Curve A. Methyl esters of fatty acids from American cockroach. Curve B. Known mixture of fatty acid methyl esters from C_8 to C_{18}. Peaks in order of appearance: S - air and solvent; C_8, caprylate; C_{10}, caproate; C_{12}, laurate; C_{14}, myristate; C_{16}, palmitate; C_{18}, stearate; $C_{18}\Delta^1$, oleate; $C_{18}\Delta^2$, linoleate; and $C_{18}\Delta^3$, linolenate.

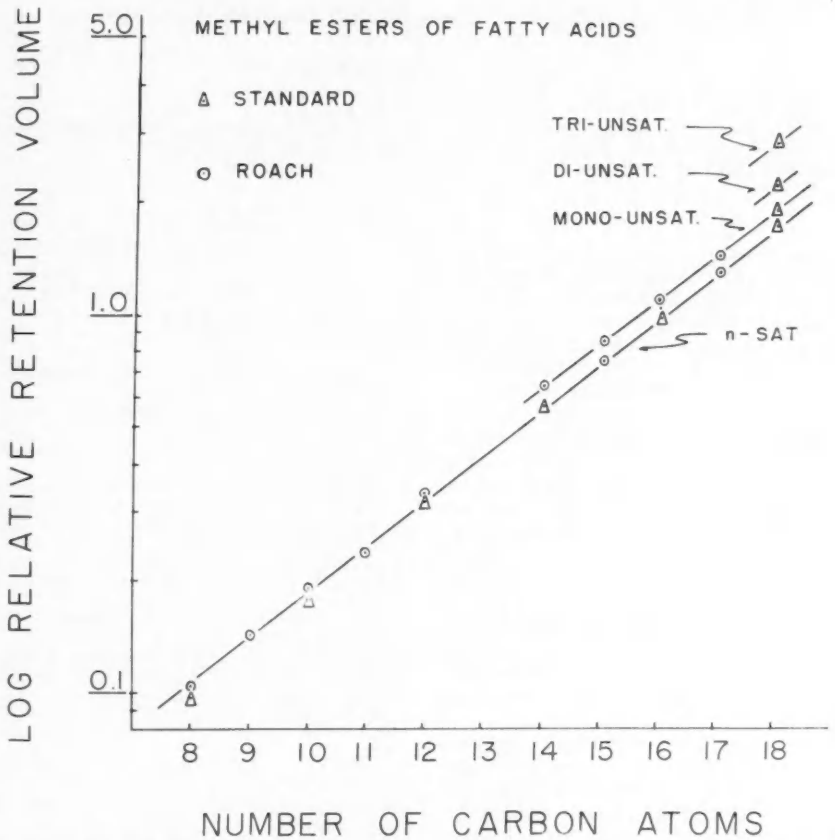

FIG. 2.—Relationship between chain-length and $\log_{10}$ of the relative retention volume (C_{16}:1) of normal saturated, mono-, di-, and tri-unsaturated fatty acid methyl esters. Where the standard and roach acids coincided, only the notation of the standard appears.

is in general agreement with the substrate preference for the fatty acid activating enzymes of this insect (Siakotos 1960).

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Collembola: Pictorial Keys to the Nearctic Genera[1]

HAROLD GEORGE SCOTT[2]

ABSTRACT

Pictorial keys are given for distinguishing 18 suprageneric categories and the 80 known Nearctic genera of Collembola. Alphabetically arranged lists name the authors of these various taxa, and give the original bibliographic reference for each one.

Public health biologists are often responsible for teaching animal and plant identification to personnel (sanitarians, engineers, physicians, veterinarians, etc.) without special training in taxonomy. One of the most successful devices for such training has been the pictorial key. The first U. S. Public Health Service pictorial key was devised by Stanley B. Freeborn and Eugene J. Gerberg (1943) to guide personnel in the identification of anopheline mosquito larvae during our national malaria control program. After the Communicable Disease Center was founded (1946), additional keys were developed, largely by Harry D. Pratt. At present, the Center utilizes 17 pictorial keys to insects, arachnids, and roundworms in its regular training program.

The plates which follow represent a further extension of this useful method, and illustrate how pictorial keys can be used to introduce the taxonomy of relatively unknown groups, and facilitate identification of specimens collected at random or during ecological surveys.

Collembola (springtail insects) are small (less than 0.3 mm. long) to medium-sized (more than 10 mm. long) arthropods subsisting primarily as scavengers. Many are capable of propelling themselves vigorously through the air by means of a caudal "spring" which, along with their small size, has given rise to such improper common names as snow fleas, garden fleas, and mushroom fleas. Eighty genera are reported from the Nearctic Realm. The most recent comprehensive paper on North American Collembola is that of Maynard (1951).

Systematic placement of Collembola is difficult. Although usually classified as insects, they differ from other insects in several important respects: (1) they have 3 pairs of abdominal appendages in the adult stage; (2) they possess only 6 abdominal segments; and (3) they exhibit holoblastic cleavage of a telolecithal egg.

Collembola are of special interest to biologists because they are the commonest soil insects (Pearse, 1946, and others), they are the oldest known fossil insects (Hirst and Maulik, 1926), they seem to have been morphologically stable through long periods of geological time, and they occupy several ecological niches otherwise devoid of insect life (Folsom, 1916).

[1] Accepted for publication June 28, 1960.

[2] Scientist Officer, Training Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta 22, Georgia. Illustrations are by C. J. Stojanovich.

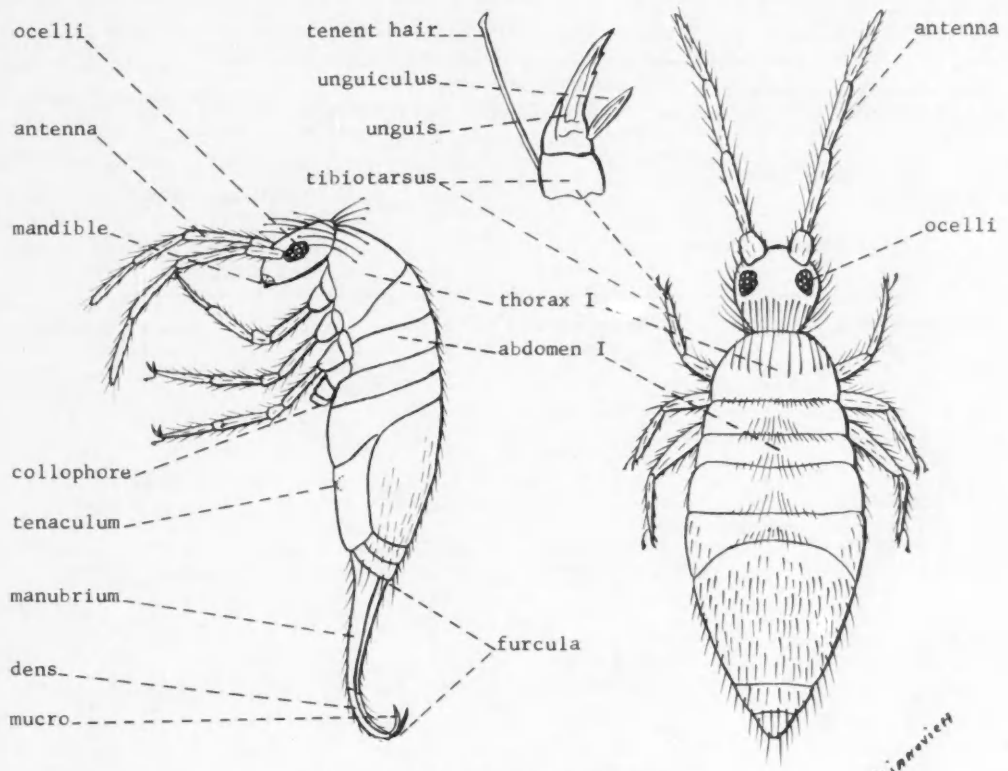
ALPHABETICAL LIST OF TAXA

SUPERGENERIC CATEGORIES

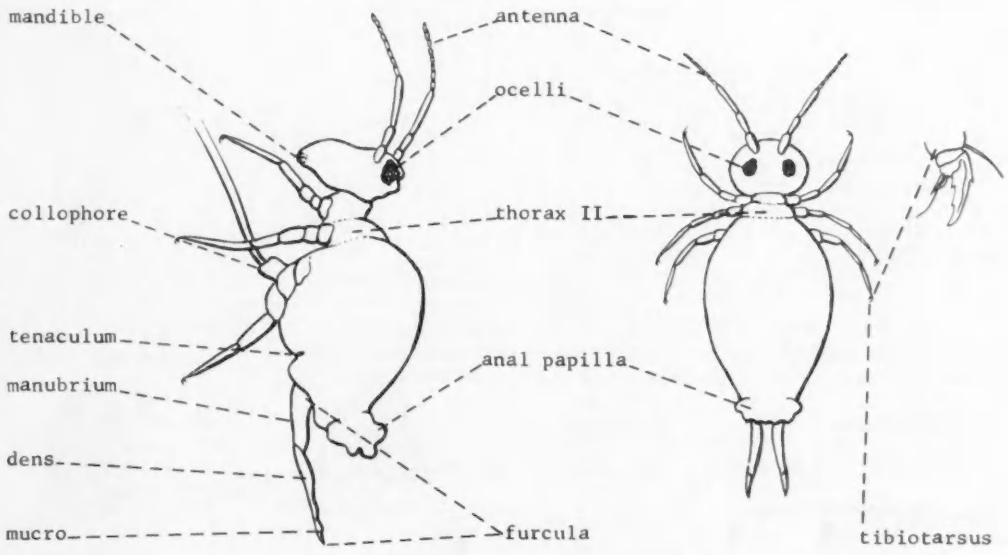
Arthropleona Börner, 1901, Zool. Anz. 24: 2.
Collembola Lubbock, 1870, Trans. Linn. Soc. London 27: 277.
Dicyrtominae Börner, 1906, Mitt. naturhist. Mus. Hamburg 23: 180.
Entomobryidae Tomösvary, 1882 Math. Term. Közlem, Magyar Akad. 18: 119.
Entomobryinae Schäffer, 1896, Mitt. naturhist. Mus. Hamburg 13: 151.
Hypogastrurinae Börner, 1906, Mitt. naturhist. Mus. Hamburg 23: 156.
Isotominae Schaffer, 1896, Mitt. naturhist. Mus. Hamburg: 179.
Neanurinae Börner, 1901, Zool. Anz. 24: 422.
Neanurini Börner, 1901, Abh. naturw. Vereins (Bremen) 17: 56.
Neelinae Handlirsch, 1929, *In*: Kükenthal's Handbuch der Zoologie, 4: 608.
Onychiurinae Börner, 1901, Zool. Anz. 24: 697.
Poduridae Lubbock, 1870, Trans. Linn. Soc. London 27: 277.
Podurinae Börner, 1906, Genera insectorum 45: 1.
Pseudachorutini Börner, 1906, Mitt. naturhist. Mus. Hamburg 23: 156.
Sminthuridae Lubbock, 1862, Trans. Linn. Soc. London 23: 589.
Sminthuridinae Börner, 1906, Mitt. naturhist. Mus. Hamburg 23: 149.
Sminthurinae Börner, 1906, Mitt. naturhist. Mus. Hamburg 23: 176.
Symphypleona Börner, 1901, Zool. Anz. 24: 1.
Tomocerinae Schäffer, 1896, Mitt. naturhist. Mus. Hamburg, 13: 177.

GENERA

Agrenia Börner, 1906, Mitt. naturhist. Mus. Hamburg 23: 173.
Anurida Laboulbene, 1865, Compt. Rend. Soc. Biol. Paris (4) 1: 189.
Anurophorus Nicolet, 1841, Nouv. Mem. Soc. Helvetia Sci. Nat. 6: 62.
Archisotoma Linnaniemi, 1912, Acta Soc. Sci. Fennicae 40: 118.
Arrhopalites Börner, 1906, Mitt. naturhist. Mus. Hamburg 23: 172.
Axelsonia Börner, 1907, *In*: Voeltzkov, Reise in Ostafrica 2: 147.
Biacanthella Scott, 1961, Ent. News, 72: (In press)
Bourletiella Banks, 1899, Jour. New York Ent. Soc. 5: 33.
Brachystomella Agren, 1903, Ent. Tidskr. 24: 113.
Calx Christiansen, 1959, Bull. Mus. Comp. Zool. 118: 532.
Cyphoderus Nicolet, 1841, Nouv. Mém. Soc. Helvetia Sci. Nat. 6: 31.
Dicyrtoma Bourlet, 1842, Mém. Soc. Roy. Centr. Agr. Dép. Nord, Douai, 9: 1.
Dicyrtomina Börner, 1903, Sitz. Ges. naturf. Freunde 3: 129.
Drepanocyrtus Handschin, 1925, Deutsche Ent. Ztg., p. 27.



SUBORDER ARTHROPLEONA

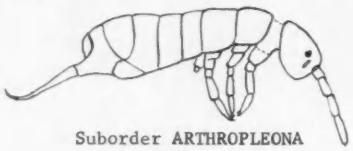


SUBORDER SYMPHYPLEONA

PICTORIAL KEY TO WORLD SUBFAMILIES OF THE ORDER COLLEMBOLA

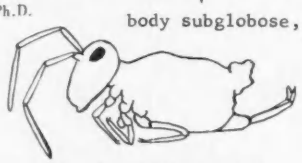
HAROLD GEORGE SCOTT, Ph.D.

body elongate, segmented



Suborder ARTHROPLEONA

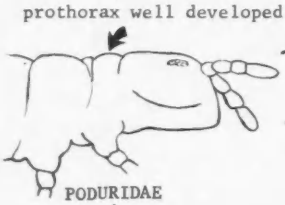
body subglobose, fused



Suborder SYMPHYPLEONA

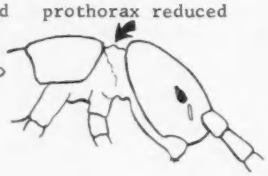
SMINTHURIDAE

prothorax well developed



PODURIDAE

prothorax reduced



ENTOMOBRYIDAE

antenna longer than head

NEELINAE

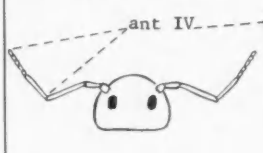
antenna shorter than head

furcula not reaching beyond collophore

PODURINAE

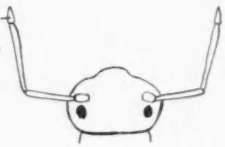
furcula reaching beyond collophore

ant IV subequal to or longer than III



DICYRTOMINAE

ant IV shorter than III

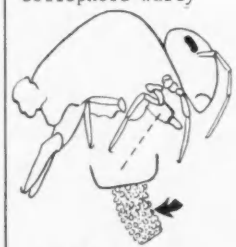


ant III sense organ with rods and cones eyes absent

ONYCHIURINAE

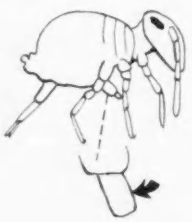
ant III sense organ with rods only eyes usually present

thoracic sutures indefinite collophore warty



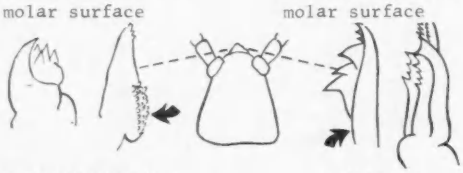
SMINTHURINAE

thoracic sutures evident collophore smooth



SMINTHURIDINAE

mandible with molar surface



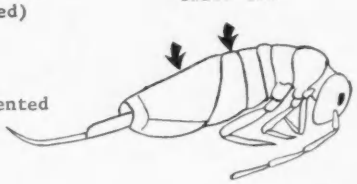
HYPOGASTRURINAE

mandible without molar surface

NEANURINAE

abd IV not twice III (or terminal segments fused)

abd IV at least twice III



ENTOMOBRYINAE

ant III and IV subsegmented

TOMOCERINAE

ant III and IV not subsegmented

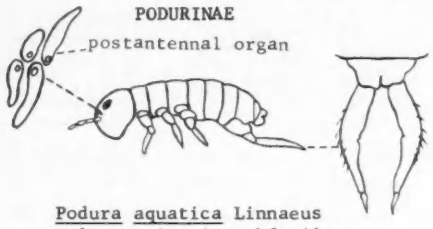
ISOTOMINAE

PICTORIAL KEY TO GENERA OF NEARCTIC COLLEMBOLA

SUBFAMILIES PODURINAE, HYPOGASTRURINAE, AND ONYCHIURINAE

HAROLD GEORGE SCOTT, Ph.D.

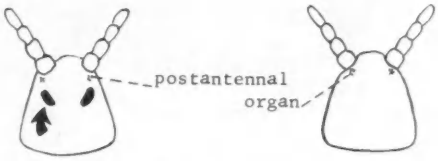
PODURINAE



Podura aquatica Linnaeus
only species in subfamily

HYPOGASTRURINAE

eyes present — eyes absent



postantennal tubercles 4-12 — postantennal tubercles 1

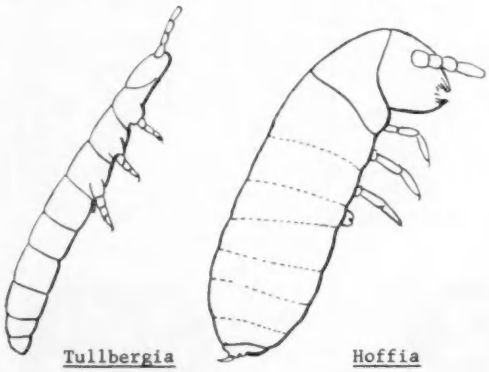
Willemia — *Stachiomella*

ONYCHIURINAE

unguiculus well developed — unguiculus reduced or absent

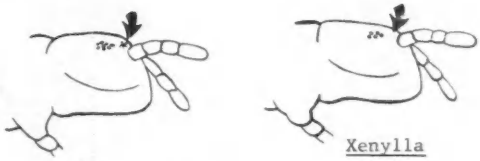
Onychiurus

body slender — body stout



Tullbergia — *Hoffia*

postantennal organ present — postantennal organ absent



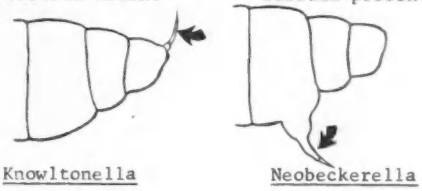
Xenylla

postantennal tubercles 4-8 — postantennal tubercles 1

eyes 2 and 2 — eyes 8 and 8

Mesachorutes — *Hypogastrura*

anal spines present furcula absent — anal spines absent furcula present



Knowltonella — *Neobeckerella*

PICTORIAL KEY TO GENERA OF NEARCTIC COLLEMBOLA

SUBFAMILY NEANURINAE

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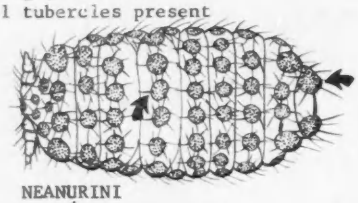
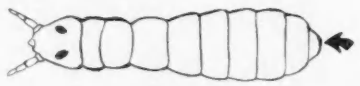
abd VI large, bilobed
segmental tubercles present



abd VI small, rounded
segmental tubercles absent



NEANURINI

PSEUDACHORUTINI

segmental tubercles large

segmental tubercles small

anal spines present

anal spines absent

furcula present

furcula absent

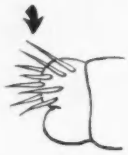
Neanura

Neanurodes

anal spines 2

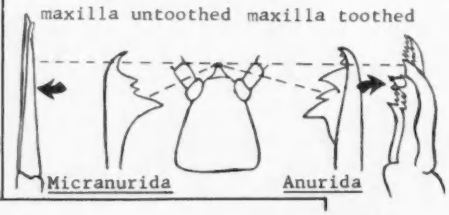
anal spines 3

anal spines 8



maxilla untoothed

maxilla toothed



Xenyllodes

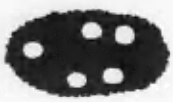
Friesea

Prospinanura

Micranurida

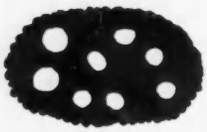
Anurida

eyes 5 and 5



eyes 6 and 6

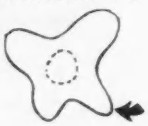
eyes 8 and 8



Microgastrura

postantennal tubercles 8

postantennal tubercles 4-5



with buccal cone

without buccal cone

Logacanura

Odontella

Brachystomella

Pseudachorutes

PICTORIAL KEY TO GENERA OF NEARCTIC COLLEMBOLA

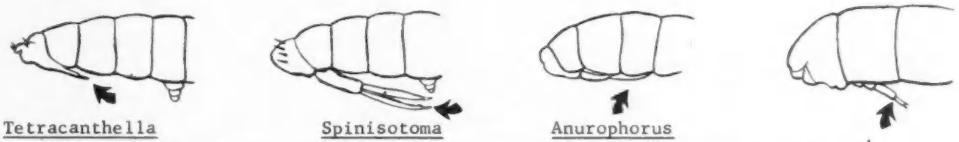
SUBFAMILY ISOTOMINAE - Part A

HAROLD GEORGE SCOTT, Ph.D.

anal spines 4 — anal spines 8 — anal spines 0-2

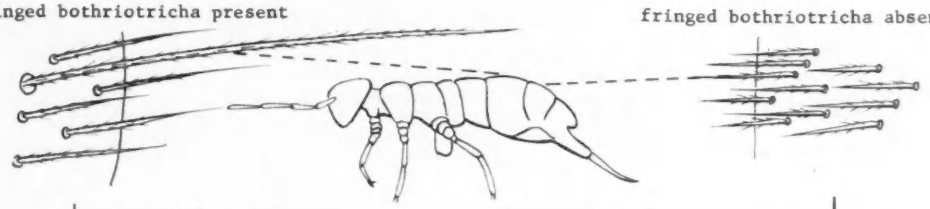
Weberacantha

furcula short — furcula long — furcula absent — furcula present

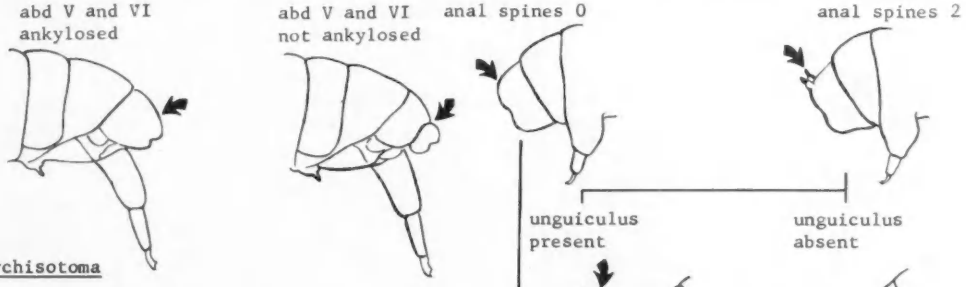


Tetracanthella — Spinisotoma — Anurophorus

fringed bothriotricha present — fringed bothriotricha absent

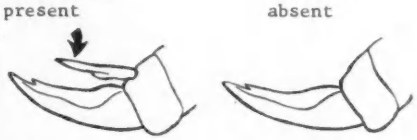


abd V and VI ankylosed — abd V and VI not ankylosed — anal spines 0 — anal spines 2



Archisotoma

unguiculus present — unguiculus absent



mucro with 3-4 teeth — mucro with 5 teeth

Biacanthella — Uzelia

Isotomurus — Axelsonia

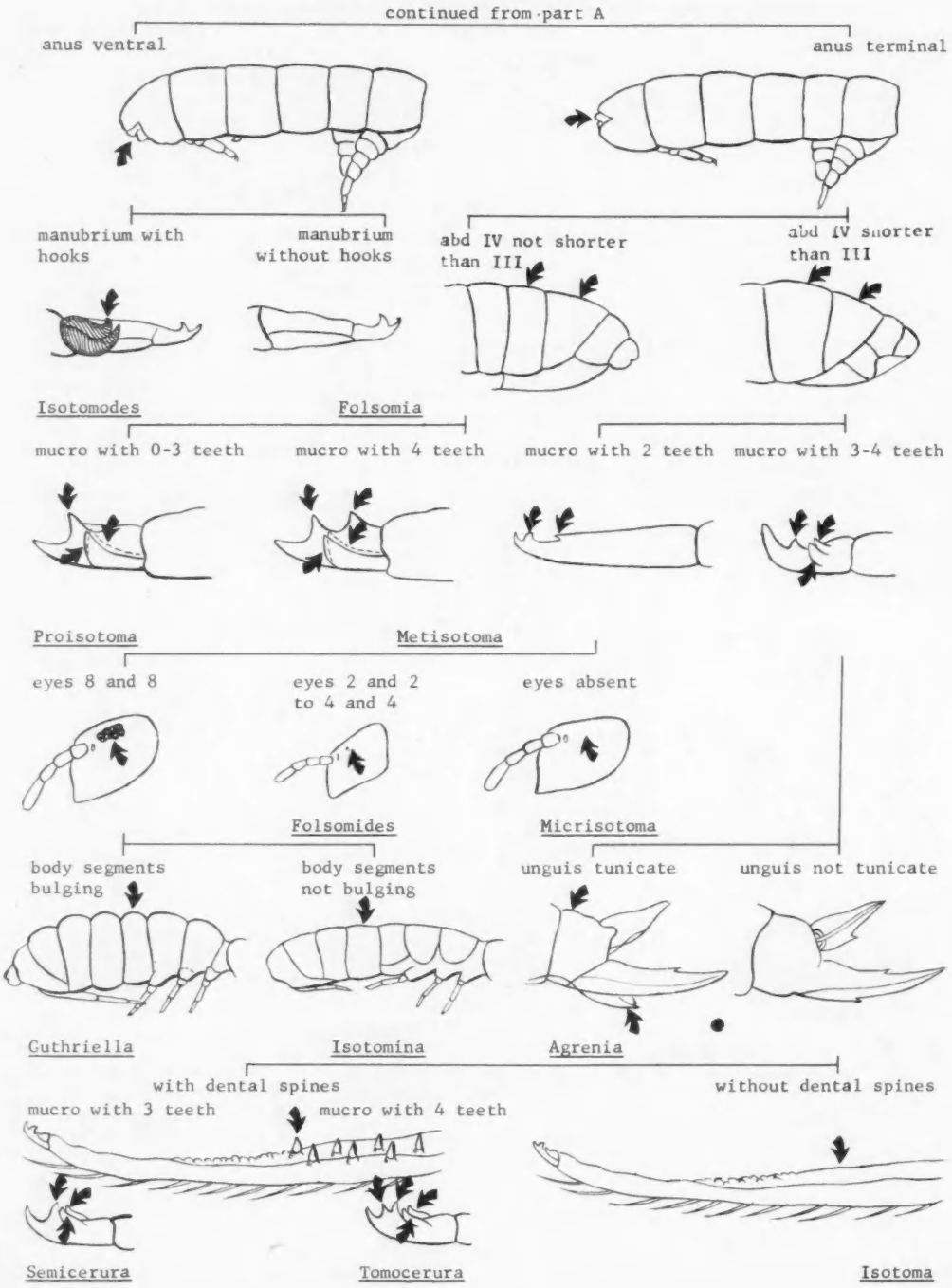
postantennal organ and eyes absent — postantennal organ present eyes present or absent

Folsomina

see part B

PICTORIAL KEY TO GENERA OF NEARCTIC COLLEMBOLA

SUBFAMILY ISOTOMINAE - Part B

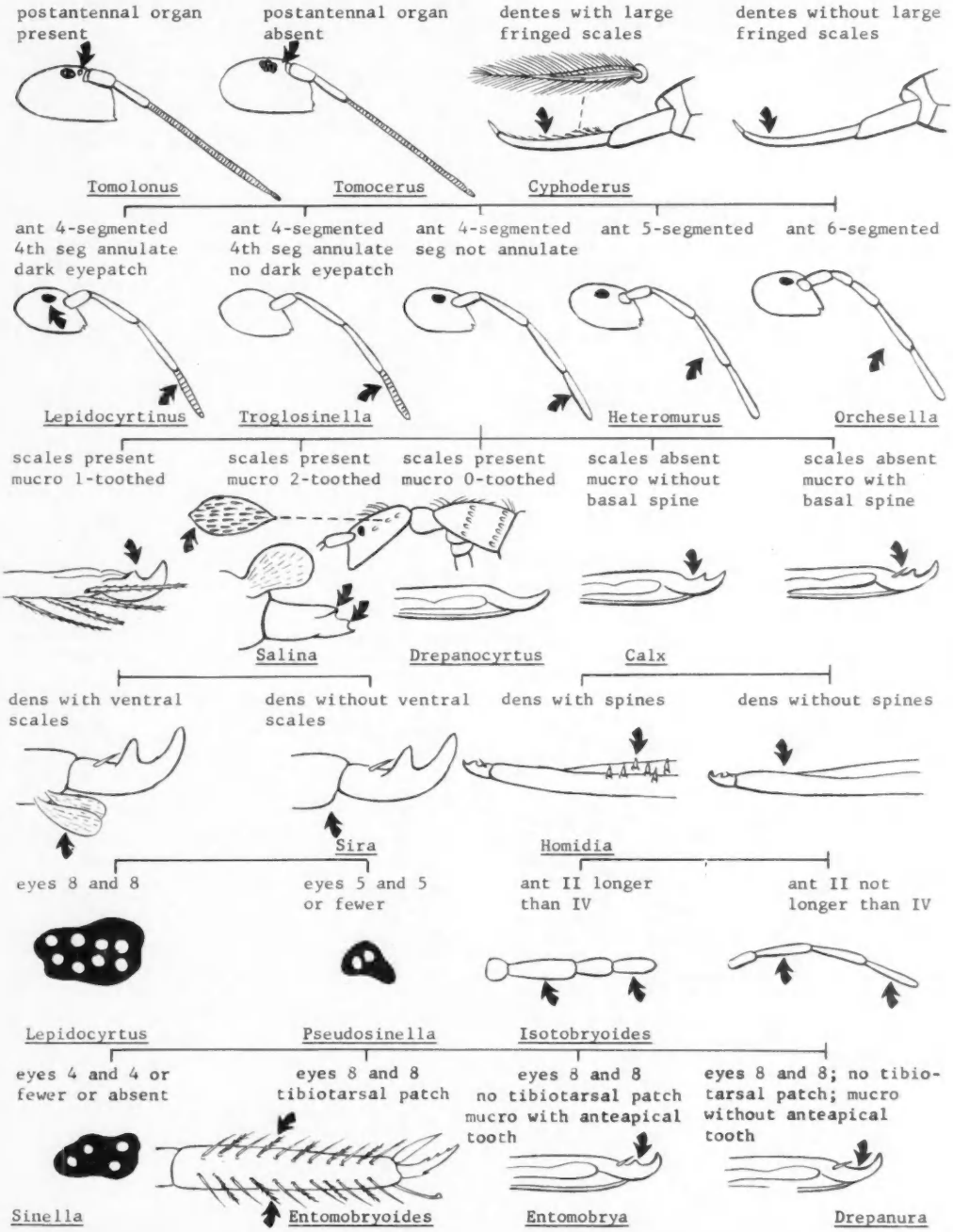


PICTORIAL KEY TO GENERA OF NEARCTIC COLLEMBOLA

SUBFAMILIES TOMOCERINAE AND ENTOMOBRYINAE

HAROLD GEORGE SCOTT, Ph.D.

TOMOCERINAE — ENTOMOBRYINAE



PICTORIAL KEY TO GENERA OF NEARCTIC COLLEMBOLA

FAMILY SMINTHURIDAE

HAROLD GEORGE SCOTT, Ph.D.

NEELINAE

ant III longer than IV

Neelides

ant III not longer than IV

Neelus

ant III and IV fused

Megalothorax

DICYRTOMINAE

claw with tunica

claw without tunica

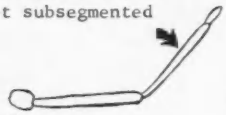
SMINTHURIDINAE

abd V and VI ankylosed

abd V and VI separated

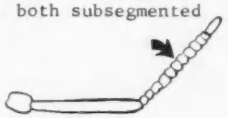
Dicyrtomina

ant III and IV not subsegmented



Dicyrtoma

ant III or IV or both subsegmented

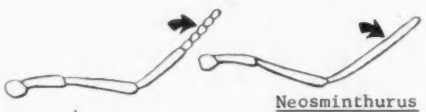


Ptenothrix

SMINTHURINAE

ant IV subsegmented

ant IV not subsegmented

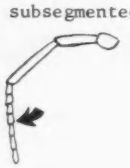


Neosminthurus

Sminthurides

ant IV not subsegmented

ant IV subsegmented



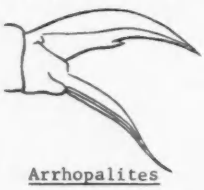
Sminthurinus

ant III with strong setae

ant III without strong setae

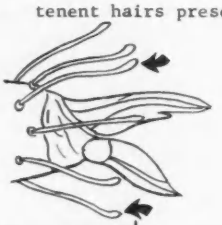
Sminthurus

tenent hairs absent



Arrhopalites

tenent hairs present

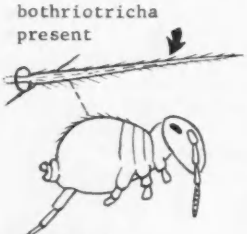


0-1 tenent hairs

several tenent hairs

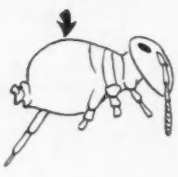
Bourletiella

bothriotricha present



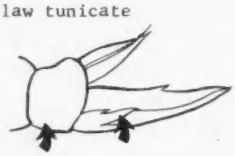
Katiannina

bothriotricha absent



Metakatianna

claw tunicate



Sphyrotheca

claw not tunicate

Pararrhopalites

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The Incorporation of 2-C^{14}-Mevalonate into House Fly Lipids[1]

J. N. KAPLANIS, R. C. DUTKY, AND W. E. ROBBINS

Entomology Research Division, Agric. Res. Serv., U.S.D.A., Beltsville, Md.

ABSTRACT

When 2-C^{14}-mevalonate was injected into male and female house flies (*Musca domestica* L.) at 10 micrograms per fly, about the same amount of radioactivity was incorporated into the saponifiable and unsaponifiable lipids after 18 hours. Fractionation of the unsaponifiable material by column chromatography demonstrated that less than 17% of the radioactive material behaved as hydrocarbons, and more than 40% was eluted in the sterol fraction. However, when this fraction was analyzed by digitonin precipitation, only trace amounts of radioactivity were precipitated with the sterol digitonides, indicating that the previously reported absence of sterol synthesis from C^{14}-acetate in the adult house fly is not due to a metabolic block in the biosynthetic pathway between acetate and mevalonate.

Mevalonic acid (3,5-dihydroxy-3-methylvaleric acid) has been shown in yeast and mammals to be an efficient precursor for the hydrocarbon squalene, which in turn is an intermediate in sterol biosynthesis (Ciba 1959). In a previous study with house flies (*Musca domestica* L.), no significant incorporation of 1-C^{14}-acetate in the sterol fraction was detected, but an appreciable amount of the radioactivity was found in the hydrocarbon fraction (Robbins et al. 1960).

This study was undertaken to determine if C^{14}-mevalonate will serve as a more efficient precursor for the house fly hydrocarbons and whether the absence of sterol synthesis in this insect may be the result of a metabolic block in the biosynthetic pathway between acetate and mevalonate.

MATERIALS AND METHODS

Two-day-old house flies from a normal strain were anesthetized with carbon dioxide and injected in the thorax with 1 microliter of an aqueous solution of the N,N′-dibenzylethylenediamine salt of 2-C^{14}-mevalonic acid at the rate of 10 micrograms per fly. After 18 hours, groups of 100 flies of either sex were homogenized in an all-glass homogenizer and saponified under nitrogen for 4 hours with 12.5% ethanolic potassium hydroxide. The unsaponifiable and saponifiable lipids were extracted with diethyl ether and pentane, respectively. The extracts were then washed with water, dried over anhydrous sodium sulfate, adjusted to a known volume with solvent, and aliquots radioassayed. No further analysis was made on the saponifiable fraction.

The unsaponifiable material was fractionated by chromatography on a 1.2 x 7.5 cm. column of 7.5 grams of Woelm acid aluminum oxide grade I, deactivated with 1.5% water, and eluted successively with 100 ml. of *n*-hexane, benzene, diethyl ether, and methanol. The hydrocarbons are eluted with *n*-hexane and the neutral sterols, including cholesterol, with diethyl ether. The radioactive hydrocarbons from male and female flies were pooled and analyzed for squalene by cochromatography with authentic squalene on a column of Woelm neutral aluminum oxide grade I (Clark and Bloch 1959).

The ether fraction from the column was further analyzed by digitonin precipitation (Sperry and Webb 1950). The digitonides were split by acetylation and the acetylated radioactive compounds were rechromatographed on aluminum oxide to determine if any of the radioactivity behaved as sterol acetates, which are eluted in the benzene fraction.

The 2-C^{14}-DL-mevalonic acid (N,N′-dibenzylethylenediamine salt) had an observable specific activity of 4,542 cpm per microgram and a radiochemical purity greater than 98% as determined by paper chromatography. Radioassays were made with a gas-flow proportional counter, and all samples counted at least in duplicate for a sufficient time to give a standard error of ±5%.

RESULTS

The results in table 1 show that from 1.7% to 2.6% of the radioactivity was used in the synthesis of the saponifiable and unsaponifiable fractions. There was no marked sexual difference in the incorporation of C^{14}-mevalonate into either fraction. About 11% and 16% in the unsaponifiable material behaved as hydrocarbons when analyzed by column chromatography (table 2), representing 0.2% and 0.4% of the injected C^{14}-mevalonate in the males and females, respectively. The sterol fractions accounted for approximately 1% of the injected material in both sexes. However, further analysis by digitonin precipitation indicated that only about 0.51% of the radioactivity present in this fraction, or about 0.005% of the applied dose was precipitated with the sterol digitonides in both the males and females (table 3). When the digitonides were pooled, cleaved by acetylation, and

Table 1.—Incorporation of injected 2-C^{14}-mevalonate into the saponifiable and unsaponifiable fractions of adult house flies.

Fraction	Percent of injected dose	
	Males[a]	Females[b]
Saponifiable	1.7	2.4
Unsaponifiable	2.1	2.6

[a] Average of two groups of 100 flies.
[b] Average of four groups of 100 flies.

[1] Accepted for publication July 8, 1960.

Table 2.—Column chromatographic analysis of unsaponifiable fraction from adult house flies injected with 2-C^{14}-mevalonate.

Fraction	Percent of radioactivity[a]	
	Males	Females
n-Hexane (hydrocarbons)	10.9	16.1
Benzene	12.4	16.8
Ether (sterols)	49.6	40.1
Methanol	10.8	10.4

[a] Averages of two groups of 100 males and four groups of 100 females. Recoveries of radioactivity from the column ranged from 75%–91%.

the acetates subjected to column chromatography on Woelm acid aluminum oxide, approximately 64% of the radioactivity was in the benzene fraction, which elutes sterol esters, and the remainder in the ether.

Table 3.—Digitonin precipitation of radioactive compounds from the sterol fraction of house flies injected with 2-C^{14}-mevalonate.

	Ether fraction (cpm)	Digitonides	
		Total cpm	Percent of total dose
Males	9.90×10^4	5.03×10^2	5.54×10^{-3}
Females	1.01×10^5	5.34×10^2	5.87×10^{-3}

Chromatographic analyses of the insect hydrocarbons demonstrated that 94% of the recovered radioactivity was in the first 50 ml. of hexane and about 6% in benzene which elutes squalene (table 4).

Table 4.—Column chromatographic analysis of hydrocarbon fraction from house flies injected with 2-C^{14}-mevalonate.

Fraction	Total cpm	Percent
n-Hexane[a]		
1	12,810	24.8
2	25,880	50.2
3	1,280	2.5
4	670	1.3
5	420	0.8
Benzene[b]		
1	2,525	4.9
2	26	0.1
3	80	0.2
4	26	0.1

[a] 10 ml. fractions.
[b] 25 ml. fractions.

When the benzene fraction was rechromatographed by this method, about 80% of the radioactive compounds were recovered, and all of these were in the benzene fraction.

DISCUSSION

Although the house fly used C^{14}-mevalonate for hydrocarbon synthesis, this compound was not as efficient a precursor as 1-C^{14}-acetate, where more than 80% of the radioactivity in the unsaponifiable lipids behaved as hydrocarbons (Robbins et al 1960). Both sexes incorporated mevalonate into the saponifiable fraction, with a much smaller difference between sexes than that found previously for 1-C^{14}-acetate. In mammals mevalonate has been reported to serve as a precursor for branched-chain fatty acids (Ciba Foundation 1959). The radioactivity in these lipids may not even be due to direct incorporation of either the mevalonate or to its five-carbon derivative the isopentoyl unit, but may represent smaller labeled moieties formed through a more extensive metabolism of mevalonate.

The small amount of radioactive hydrocarbons eluted with squalene does not indicate the presence of C^{14}-squalene, as the house fly hydrocarbon(s) has been found to be a complex mixture of compounds (Louloudes, unpublished data), and this radioactivity may simply represent a minor component that behaves chromatographically like squalene (Clark and Bloch 1959).

Although considerably more mevalonate than acetate was incorporated into the sterol fraction, only a small percentage of this was digitonin-precipitable. Mevalonate has been shown to be very efficiently incorporated into sterols both in vivo (Garattini et al. 1959) and in vitro (Tavormina and Gibbs 1956) by organisms capable of sterol biosynthesis. Thus the extremely low incorporation of C^{14}-mevalonate into house fly sterol digitonides eliminates the possibility that the absence of sterol synthesis from C-^{14}-acetate in this organism is due to a block between acetate and mevalonate. The low levels of radioactivity in the digitonides may be due to the synthetic activity of associated microorganisms (Pant and Fraenkel 1954), and/or may represent non-steroidal C^{14} compounds precipitated by digitonin (Fieser and Fieser 1949). Most of these radioactive compounds did behave as alcohols in that they were eluted in a different chromatographic fraction following acetylation, but the levels of radioactivity were so low that further analysis was impractical. Clark and Bloch (1959) have reported the presence of a long-chain C^{14}-labeled aliphatic alcohol in *Dermestes vulpinus* larvae following feeding of C^{14}-acetate.

The lack of any appreciable incorporation of C^{14}-mevalonate into house fly sterols is in keeping with prior biochemical data obtained with 1-C^{14}-acetate (Robbins et al. 1960) and the nutritional finding that adult house flies require a dietary source of sterols for sustained normal reproduction (Monroe 1959, 1960).

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Autogeny in Panamanian *Phlebotomus* Sandflies (Diptera: Psychodidae)[1]

PHYLLIS T. JOHNSON

Gorgas Memorial Laboratory, Panamá, República de Panamá

ABSTRACT

In the course of leishmaniasis studies at Gorgas Memorial Laboratory females of *Phlebotomus gomezi* Nitz., one of the laboratory-reared species, occasionally were discovered producing fertile eggs without a previous blood meal. Nine autogenous strains of this species, one reaching the 11th generation, have been maintained in laboratory culture. Autogenous females do not need any form of food as adults and apparently produce eggs by utilizing the well-developed fat body, which never shows comparable development in normal, anautogenous females. Normal strains of *P. gomezi*, some now in the 13th laboratory generation, have never been observed to reproduce autogenously in the laboratory. Attempts at reciprocal crossing indicate that autogenous and normal individuals of the laboratory strains do not interbreed, or at least do not produce fertile eggs. Autogenous females are found in nature in small numbers throughout the year.

The production of viable eggs without a previous blood meal by a bloodsucking insect was first noted in the mosquito *Culex pipiens* by Roubaud and Toumanoff (1930), a phenomenon for which they proposed the term *autogeny*. To date autogeny has been reported to occur in several dipteran species representing various members of the Culicidae and the genera *Culicoides, Simulium*, and in *Phlebotomus papatasi* Scop. from Russia. It is quite possible that autogeny occurs in many more bloodsucking dipteran species but it would be difficult if not impossible to demonstrate its occurrence in a wild population, and even during the process of laboratory rearing it might be overlooked unless a special search were undertaken.

The present paper constitutes a report of autogeny in a Panamanian species of *Phlebotomus*. In connection with leishmaniasis studies at Gorgas Memorial Laboratory, several species of Panamanian *Phlebotomus* sandflies have been established and maintained in successive laboratory cultures. One of these species, *P. gomezi* Nitz., occasionally lays fertile eggs without a previous blood meal. We have maintained nine autogenous strains of this species, the oldest having passed through 11 laboratory generations. Each strain was from a different female or pool of two to five females, at least one of which was autogenous.

MATERIALS AND METHODS

The autogenous and anautogenous, or normal, strains of *gomezi* in our laboratory were all derived from wild-caught females which had recently fed on man or horse, or were gravid when collected from resting places in the jungle. Wild-caught females were maintained separately in large, moistened, plaster-lined vials, in which they oviposited. Eggs from those females identified as *gomezi* were left for rearing in the original vials, or two or more batches of eggs were transferred to our standard breeding vessel, a plaster-lined earthenware pot.

Larval food consisted of autoclaved, dried and ground rabbit feces which had been soaked in human blood, or of an autoclaved mixture of ground rabbit feces, decaying forest leaves and dried insects.

Adults of the first laboratory generation (progeny of the wild-caught females) and succeeding laboratory generations were allowed access to a boiled raisin; females of the normal strain were allowed a blood meal.

CHARACTERISTICS OF THE AUTOGENOUS STRAIN

Males and females of the autogenous and normal

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strains have been compared by Dr. G. B. Fairchild, of our staff. He has been unable to find any morphological differences between the two strains.

In comparing the length of life cycle of autogenous and normal strains the only difference seems to be a lengthening of the egg stage. Of 81 normal females, 66 laid eggs which hatched in 6 to 8 days; 15 produced eggs which hatched in 9 to 10 days, while eggs from two females did not hatch until 11 and 12 days, respectively. Of five autogenous females, two laid eggs which hatched in 8 days, one in 10 days, one in 11 days, and one only after 14 days.

Autogenous females will suck blood both in nature and in the laboratory. Of the nine autogenous strains we have had in the laboratory at various times, all but one came from eggs produced by females known to have had a blood meal. That blood was not necessary for egg production seems indicated by the fact that four of the nine females laid eggs only 3 days after the blood meal. It is probable that egg development was already under way at the time of feeding, since normal *gomezi* rarely produce eggs in less than 4 days and usually take 5 to 8 days under laboratory conditions.

On the average, autogenous females produce fewer eggs than do normal ones. Most normal females lay from about 15 to 40 eggs, with extreme ranges of from 1 to 70 (based on 208 observations). Fourteen autogenous females laid from 4 to 22 eggs.

The dissection of 40 autogenous females and over 200 normal females, ranging in age from newly emerged to 72 hours, has revealed one constant difference between the two groups. The autogenous females invariably have a well-developed fat body at the time of emergence. Normal *gomezi* females never have a fat body which is discernible with the dissecting microscope. Dolmatova (1946), who was the first to observe autogeny in a species of *Phlebotomus*, did not mention the relative development of the fat body in her series of autogenous and normal females. She did observe that of eight laboratory-reared, unfed *papatasi* females, from eastern Georgia, two laid viable eggs autogenously and furthermore, 18 of 21 laboratory-reared, unfed females underwent development to varying degrees of the ovaries.

Females of the autogenous strains need no source of food as adults in order to lay viable eggs. The larval food given in the laboratory is a mixture rich in nitrogenous material, particularly in the case of the food with added blood. However, it appears that autogenous development in *gomezi* is not a response to the concentrated food. Our normal strains, some of which are now in the 13th laboratory generation, have been maintained on the same diet as the autogenous strains and have never been observed to reproduce autogenously.

THE FREQUENCY OF AUTOGENY IN WILD POPULATIONS

Some of our autogenous strains came from an experimental series undertaken with the express purpose of obtaining some indication of the incidence of autogeny in *gomezi* taken in our routine field collections. A large number of such wild-caught females were kept separately from the time they arrived in the laboratory. Over a period of nearly a year, 11 June 1959 to 8 May 1960, a total of 113 wild-caught *gomezi* females, all of which had taken a blood meal from man or horse in the field, laid eggs in their individual plaster-lined vials. The eggs hatched and the larvae, with blood-soaked rabbit feces as food, were reared to the adult stage. These adults were kept in their original vials, without access to any blood meal, until all had died. The vials were then examined for the presence of eggs or young larvae. The absence of either eggs or larvae was taken as an index of a normal strain.

Of the 113 females, 5 (4.4 percent) produced autogenous offspring. The dates of collection of these five were 16 June, 24 November, 1959, 8 February, 7 and 14 March, 1960. For the calendar months in which autogenous females were found, there were 1 autogenous of 2 females collected in June, 1 of 2 in November, 1 of 50 in February, and 2 of 46 in March. The rest of the 113 females were scattered throughout other months. Two areas in the Canal Zone and one in the Republic of Panama were represented. While our samples are small, there is no obvious correlation between the time of year or locality of collection and the proportion of autogenous to normal females.

ATTEMPTS TO CROSS AUTOGENOUS AND NORMAL STRAINS

Various workers have found that strains of autogenous and anautogenous *Culex pipiens* are interfertile (Weyer 1936, Rozeboom and Gilford 1954). In order to ascertain whether the same is true of our *P. gomezi* strains the following tests were made.

Pupae of an autogenous and a normal strain, both of the seventh laboratory generation, were maintained individually until adult emergence to assure a supply of virgin males and females. Upon emergence a male of one strain and female of the other were placed together in a rearing vial. Crosses of 9 autogenous ♂♂ x 9 anautogenous ♀♀ and of 14 anautogenous ♂♂ x 14 autogenous females were made. The females of the normal strain were allowed a blood meal, and all members of the series had access to a boiled raisin. Eggs were laid by all the females, both autogenous and normal. None of the eggs hatched. Controls consisted of a paired male and female of each of the two strains. They were maintained under conditions similar to the test series except that the autogenous pair did not have access to a raisin. Both control females laid eggs which hatched into healthy larvae.

To test whether the lack of interstrain fertility might be caused by the reluctance of mixed strains to mate in a small container, a second series was carried out, utilizing rearing pots rather than vials. Ninth generation males and females from isolated

pupae and of the same autogenous strain as above, but of a different normal strain, were placed together in reciprocal crosses in two rearing pots. Seven autogenous ♂♂ x three fed anautogenous ♀♀ produced 58 eggs, none of which hatched. Eight anautogenous ♂♂ x thirteen autogenous ♀♀ produced 135 eggs, none of which hatched.

It has not been determined whether the cause of interstrain sterility is lack of mating or physiological incompatibility. Two methods of demonstrating mating would be either to observe pairs in copula or to find sperm in the spermathecae. I have never observed *P. gomezi*—either autogenous or normal—in copula in the laboratory. *Gomezi* females of both the normal and autogenous strains which had laid fertile eggs, and hence had mated, nevertheless rarely contained sperm in the spermathecae when dissected. Of 3 autogenous and 19 normal females of varying ages, with males continuously present, none of the autogenous and only two of the normal females had sperm in the spermathecae.

DISCUSSION

The role of autogeny in the population dynamics of insects which are ordinarily obligate bloodsuckers has not been ascertained. Autogeny might aid insects, such as sandflies, to maintain their populations during periods of stress, as, for example, a sudden reduction in numbers of hosts, or periods of unfavorable weather when the insect might be less able to leave its shelter to seek a blood meal, as in prolonged stormy weather or severe drouth.

Since the samples of *P. gomezi* in this study were not homogeneous and do not lend themselves to statistical analysis, it can only be said that a small percentage of females reproduce autogenously both during the rainy season, May-December, and the dry season, January-April.

At the present time we do not know whether autogeny in *gomezi*, which is apparently a genetically controlled phenomenon, is a multiple factor or blending type of inheritance, as may be the case in *C. pipiens* (Spielman 1957). The presumptive laboratory evidence for lack of interbreeding between established autogenous and anautogenous strains points to the possibility that the genetic basis for autogeny in *gomezi* may be different from that found in *C. pipiens*. Further experiments involving wild-caught females with a heterogeneous gene make-up will be necessary before more can be said regarding the genetics of *P. gomezi* autogeny.

It is not known whether the phenomenon of autogeny occurs throughout the entire range of *gomezi*, which is from Nicaragua to the Amazon. Dolmatova (1946), working with *Phlebotomus papatasi* from two areas in Tadzhikistan and one area in eastern Georgia, observed autogeny in the Georgian population but none in populations from the two areas in Central Asia.

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Water Mites of the Genus *Feltria* in Central and Western United States (Acarina: Feltriidae)[1]

DAVID R. COOK

Wayne State University, Detroit, Michigan

ABSTRACT

Descriptions and figures are given for 16 species of *Feltria* known from central and western United States, including 11 that are new: *F.* **polyplacophora,** *F.* **multiscutata,** *F.* **rubra nearctica,** *F.* **montanensis,** *F.* **major,** *F.* **lundbladi,** F. **curviseta,** *F.* **oregonensis,** *F.* **flatheadensis,** *F.* **parva,** and *F.* **laversi.** New synonymy: *F. macroplata geometrica* Habeeb (= *F. occidentalis* Habeeb). A schematic drawing of the basic dorsal plates and glandularia is given.

This paper deals with sixteen species of *Feltria* collected in Michigan and western United States. Eleven species are described as new. Of the remaining five species, one is the holarctic species, *Feltria minuta* Koenike, and the other four appear to be *F. macroplata geographica, F. amoenella, F. purpurotincta,* and *F. rivophila* described by Habeeb (1955a) from eastern North America. Types were not designated for the latter species and the specimens apparently have been privately retained. The present

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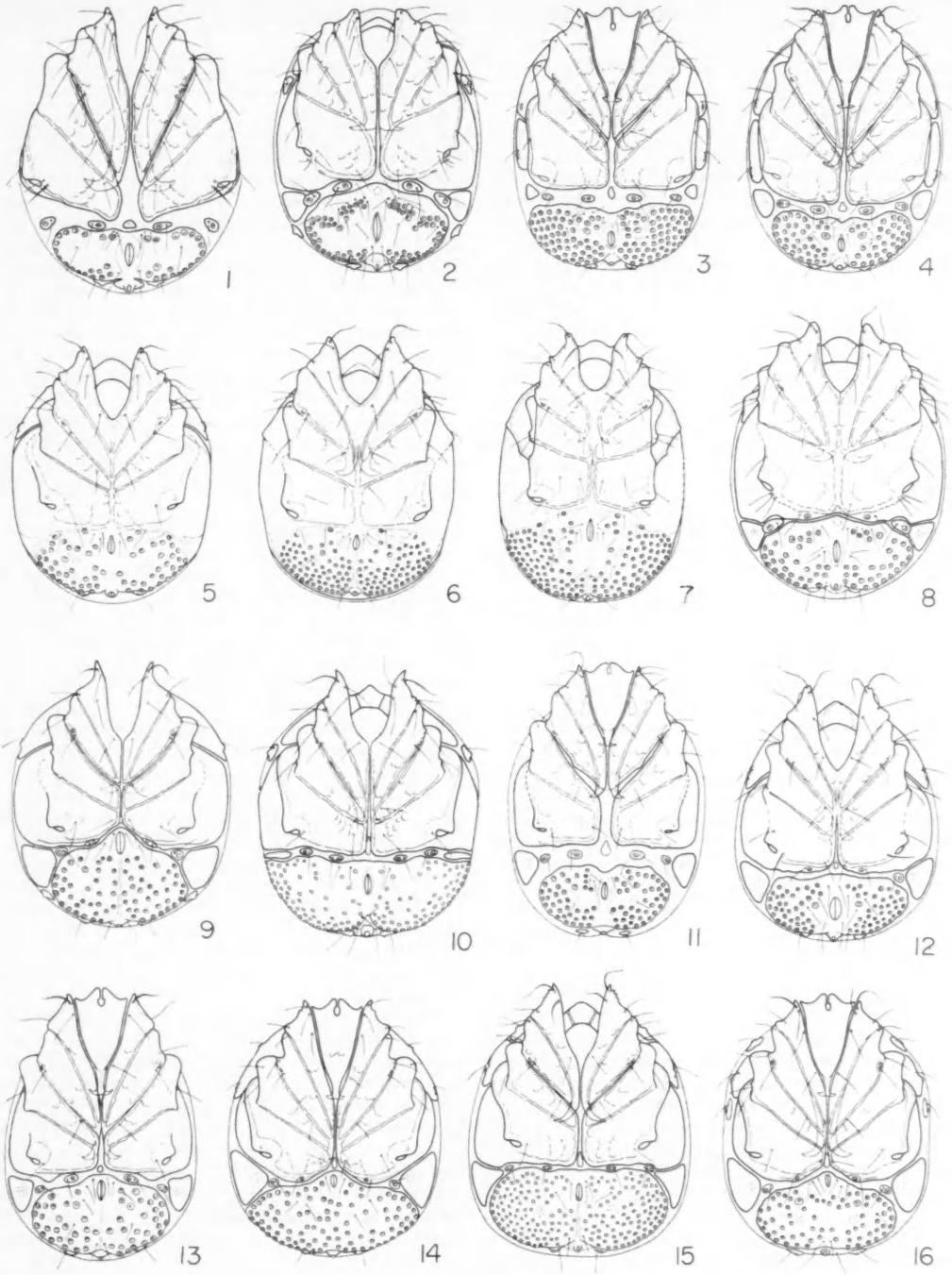


[PLATE 1]

FIGS. 1-16.—Ventral view of males. FIG. 1.—*Feltria multiscutata*, n. sp. FIG. 2.—*F. polyplacophora*, n. sp. FIG. 3.—*F. amoenella* Habeeb. FIG. 4.—*F. rubra nearctica* n. ssp. FIG. 5.—*F. lundbladi*, n. sp. FIG. 6.—*F. montanensis*, n. sp. FIG. 7.—*F. major*, n. sp. FIG. 8.—*F. macroplata geometrica* Habeeb. FIG. 9.—*F. purpurotincta* Habeeb. FIG.10.—*F. curviseta*, n. sp. FIG. 11.—*F. flatheadensis*, n. sp. FIG. 12.—*F. oregonensis*, n. sp. FIG. 13.—*F. parva*, n. sp. FIG. 14.—*F. rivophila* Habeeb. FIG. 15.—*F. minuta* Koenike. FIG. 16.—*F. laversi*, n. sp.

author requested the loan of these specimens but received no reply. Thus it has been necessary to base identifications on the rather inadequate original descriptions.

The genus *Feltria* has been divided into two subgenera, based on the chaetotaxy of III-Leg-6 in the male. Species in which the setae on the ventral surface of III-Leg-6 are small and hairlike or absent (figs. 60-64) are placed in the subgenus *Feltriella*, while species in which these setae are thickened and often variously modified (figs. 65-74, 76) are placed in *Feltria s.s.* The setae of III-Leg-6 often make excellent characters for specific identification if it is realized that they do vary somewhat in number and degree of fusion, even of opposite sides of the same individual. For example, in the species illustrated in figure 74, the number of thickened ventral setae varies from one to three. Although figures 65 and 66 represent different species, the degree of lateral fusion of the setae illustrates variation which might easily occur within the same population. The palps of most species are rather similar, the most noticeable exception being *F. macroplata geometrica*.

Unlike the condition in the majority of other water mites, the fourth segment of the palp is usually considerably broader than high. Consequently, slight variations in orientation of the palp on a slide will change considerably the *apparent* height of the segment. For this reason the palp is here used very little for specific identification, although palps of the males are illustrated. The palps of the females (not illustrated) by and large are longer and comparatively slimmer than those of the male.

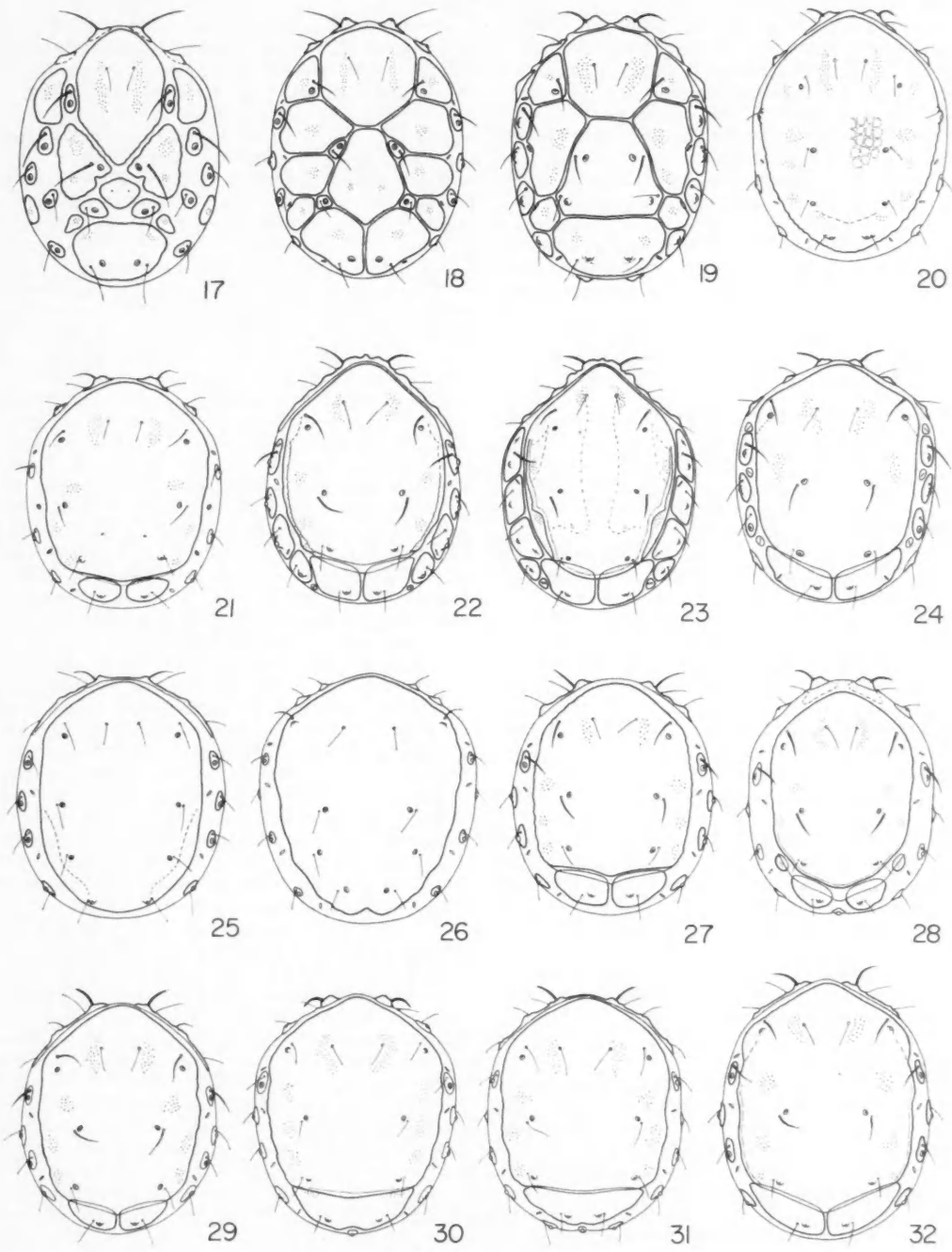
The characters most used in specific identification are shape and degree of fusion of the coxae, genital field, and especially the plates of the dorsum. The dorsum contains several pairs of glandularia and muscle attachment plates (dorsal plates) as illustrated in figure 33. Most species of *Feltria* exhibit varying degrees of fusion of these plates and glandularia, but in the female of *F. multiscutata* n. sp. (fig. 34) they may all be separate. To avoid the implication of homology (although homologies may actually exist) between the dorsal plates of *Feltria* and the Thyasinae as given by Lundblad (1927), letters rather than numbers are used to designate the plates and glandularia. It should be pointed out that the "anteromedial plate" may well have been a pair of plates in the primitive condition. In one known species, *F. menzeli* Walter, there actually are right and left plates. Also, what the present author calls Dorsoglandularia D is larger than the other glandularia and possesses muscle attachment scars. This strongly suggests that Dorsoglandularia D is made up of fused glandularia and dorsal plates. The author knows of no instance in which the two are separated and for the sake of convenience the term Dorsoglandularia D is used. Even in species where there has been extensive fusion of the dorsal plates, the muscle scars, indicated by areas of stipple on the illustrations, will aid in determining homologies. The dorsum also contains small, slitlike glands without associated setae. These glands may occur on small platelets (fig. 42) or lie free in the integument (fig. 46). The term "ventrolateral plates" has been given to a pair of muscle attachment plates lying slightly anterolateral to the acetabular plates. Usually the ventrolateral plates lie free in the integument (figs. 51-56) but occasionally may be fused with the acetabular plates (figs. 57, 58). The term length used at the beginning of each description, unless otherwise specified, refers to the distance between the anterior end of the first coxae and the posterior end of the genital field. In measurements of the genital field, length refers to the greatest distance in an anterior-posterior direction, width refers to the greatest distance from medial to lateral edges.

Holotypes and allotypes will be placed in the Chicago Natural History Museum. Paratypes, whenever possible, will be deposited in the United States National Museum.

1. **Feltria (Feltriella) polyplacophora,** new species
Figs. 2, 18, 38, 47, 61, 91

Male: Length 350μ ; coxal groups separated medially, but completely fused on their respective sides (fig. 2); apodemes from first coxae short; both pairs of glandularia between coxae and genital field free; genital field 192μ in width; genital opening near middle of genital field; genital acetabula 38-39 on each side; excretory pore fused with acetabular plates; ventrolateral plates small and triangular in shape; dorsum with plates and glandularia arranged as follows: anteromedial plate separate; dorsal plates A and dorsoglandularia A fused on their respective sides; dorsal plates D fused medially and enlarged into a centrally located, somewhat five-angled plate; all other plates and glandularia separate (fig. 18); dorsal lengths of the palpal segments, P-I 16μ, P-II 64μ, P-III 24μ, P-IV 68μ, P-V 40μ; figure 91 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 72μ; III-Leg-5, 84μ, III-Leg-6, 90μ; figure 61 shows III-Leg-6.

Female: Length 471μ-480μ; coxal groups separate (fig. 47); apodemes of first coxae very short; both pairs of glandularia between fourth coxae and genital field free; the individual acetabular plates 120μ-128μ in width, 124μ-132μ in length; genital acetabula 31-36 on each side, these arranged in an anteromedial group of 8-10, and the remainder around the lateral and posterior periphery of the acetabular plates; pregenital sclerite somewhat four-angled, and located between anteromedial portions of the acetabular plates; fusion of the dorsal plates and glandularia as in male; shape of individual plates differing slightly as shown in figure 38; dorsal lengths of the palpal segments, P-I 18μ-20μ, P-II 62μ-64μ, P-III 25μ-28μ, P-IV 66μ-70μ, P-V 44μ-48μ; P-V longer and slimmer than in male; dorsal lengths of the distal segments of the first leg, I-Leg-4, 54μ; I-Leg-5, 66μ; I-Leg-6, 80μ.



[PLATE 2]

FIGS. 17-32. Dorsal view of males. FIG. 17.—*Feltria multiscutata*, n. sp. FIG. 18.—*F. polyplacophora*, n. sp. FIG. 19.—*F. macraplata geometrica* Habeeb. FIG. 20.—*F. amoenella* Habeeb. FIG. 21.—*F. rubra nearctica*, n. ssp. FIG. 22.—*F. montanensis*, n. sp. FIG. 23.—*F. major*, n. sp. FIG. 24.—*F. lundbladi*, n. sp. FIG. 25—*F. purpurotincta* Habeeb. FIG. 26.—*F. curviseta*, n. sp. FIG. 27.—*F. oregonensis*, n. sp. FIG. 28.—*F. flatheadensis*, n. sp. FIG. 29.—*F. parva*, n. sp. FIG. 30.—*F. rivophila* Habeeb. FIG. 31.—*F. laversi*, n. sp. FIG. 32.—*F. minuta* Koenike.

Types: Holotype, adult male, taken in a small stream near Tioga Lake, Mono County, California, August 4, 1954; allotype, adult female, collected in seepage-wetted mosses on the sheer face of a cliff along State Highway 5, in northwestern Lewis County, Washington, August 13, 1954; paratypes, 2 females, same data as allotype.

Feltria polyplacophora is closely related to *F. m. macroplata* and *F. m. geographica* but differs in that there is less fusion of the dorsal plates and glandularia in the present species. The genital acetabula of *polyplacophora* are more or less confined to the periphery of the acetabular plates in contrast to the general scattering of the acetabula in *macroplata* and *geometrica*. The palp of the present species is shorter, especially P-IV, and lacks the projecting ventral side of P-II.

2. **Feltria (Feltriella) macroplata geometrica** Habeeb

Figs. 8, 19, 37, 48, 64, 90

F. *geometrica* Habeeb, 1955, *Ibid.*, No. 6: 2.
F. *occidentalis* Habeeb, 1959, *Ibid*, No. 19: 5. **(new synonymy).**

Male: Length 349μ-410μ; coxal groups completely fused; apodemes from first coxae short; medial pair of glandularia located between coxae and genital field fused with fourth coxae (fig. 8); genital field 188μ-244μ in width; genital opening small, located near middle of genital field; genital acetabula 19-32 on each side; excretory pore fused with acetabular plates; ventrolateral plates small and triangular in shape; dorsum with plates and glandularia arranged as follows: anteromedial plate separate; dorsal plates A and dorsoglandularia A fused on their respective sides; dorsoglandularia B and C and dorsal plates D fused into a large, centrally located plate (fig. 19), although in one specimen dorsoglandularia C were only partially fused; dorsal plates B and C fused on their respective sides; dorsoglandularia D broadly fused medially; dorsal lengths of the palpal segments, P-I 18μ-20μ, P-II 56μ-64μ, P-III 41μ-52μ, P-IV 92μ-108μ, P-V 40μ-46μ; ventral side of P-II with a rounded projection, often much more pronounced than shown in figure 90; dorsal lengths of the distal segments of the third leg, III-Leg-4, 68μ-84μ, III-Leg-5, 88μ-100μ; III-Leg-6, 88μ-99μ; figure 64 illustrates III-Leg-6.

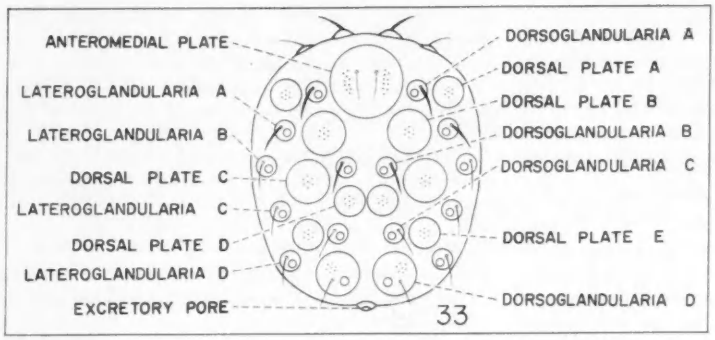
Female: Length 384μ-489μ; coxal groups separate (fig. 48) or with the first coxae fused in the midline; apodemes from first coxae very short; both pairs of glandularia between the coxae and genital field free; the individual acetabular plates 92μ-128μ in width, 84μ-136μ in length; genital acetabula 21-42 on each side; anterior edge of acetabular plate with rounded projection extending partially between glandularia; pregenital sclerite somewhat four-angled, and located between anteromedial portions of the acetabular plates; dorsal plates and glandularia arranged as follows: anteromedial plate separate, length of this plate 120μ-164μ, width 140μ-176μ; dorsal plates A and dorsoglandularia A fused on their respective sides; dorsal plates D and dorsoglandularia B fused into a single median plate; dorsoglandularia D broadly fused medially; other plates and glandularia separate (fig. 37); dorsal lengths of the palpal segments, P-I 17μ-20μ, P-II 60μ-70μ, P-III 45μ-56μ, P-IV 102μ-116μ, P-V 38μ-46μ; ventral side of P-II with a rounded projection, often much more pronounced than in male; dorsal lengths of the distal segments of the first leg, I-Leg-4, 52μ-64μ; I-Leg-5, 62μ-76μ; I-Leg-6, 68μ-84μ.

The type locality of this subspecies is Coldbrook near Grand Falls, New Brunswick. The author has taken *geometrica* in springs and cold streams in widely scattered areas in both peninsulas of Michigan (Barry, Wexford, Lake, and Alger Counties) and in a cold spring in Lake County, Montana. Habeeb (1959) described *F. occidentalis*, which is here considered a synonym of *geometrica*, from the north branch of the San Gabriel River, Los Angeles County, California.

Feltria macroplata geographica was described by Habeeb (1955a) as a distinct species. In a later paper (Habeeb 1955b), he pointed out the similarities between *geometrica* and *F. macroplata*, which was described by Imamura (1954) from Japan. Habeeb (1955b) mentioned that *geometrica* has approximately 50% more genital acetabula than the Japanese species. However, now that longer series of specimens are available from North America, this characteristic is found not to hold up. The main difference between the two forms is that in the male of *geometrica* the more medial pair of glandularia between the coxae and the genital field is fused with the fourth coxae, while in *macroplata* both pairs of glandularia are free. This is a very small difference in the light of what is known concerning variation in fusion of the plates and glandularia in the genus *Feltria*. However, since the morphological differences are correlated with distributional differences, they are here considered to be separate subspecies.

The species *F. occidentalis* was described by Habeeb (1959) and based largely on a female specimen in which the first coxae were fused medially. However, this characteristic is not of diagnostic value, as is shown by the fact that of four female specimens of *geometrica* taken in a single collection in Lake County, Montana, two specimens had the first coxae separated medially, but in the other two specimens the first coxae were fused medially as in *occidentalis*. Other characters were given, such as smaller size and fewer acetabula in the western species, but it is now known that these characters fall within the range of variation of *geometrica* from Michigan. Based on present evidence it would seem best to consider *occidentalis* as a synonym of *geometrica*.

Lundblad (1941) described a new genus and species, *Testudofeltria longipalpis*, from Burma. Although the description is very short and is without illustrations, it appears that the Burmese species is



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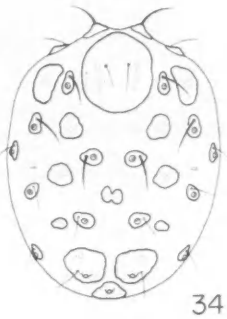

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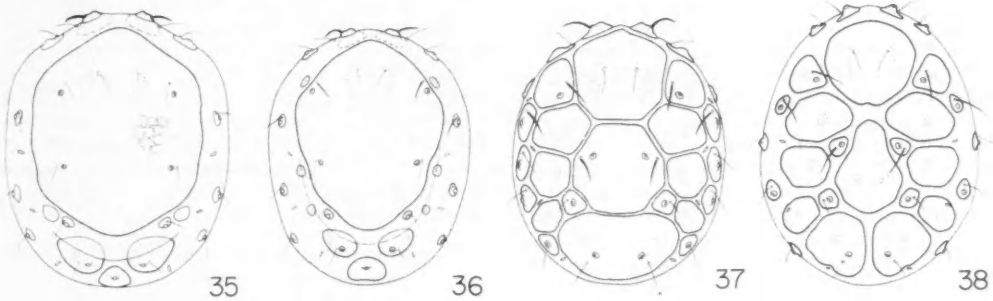

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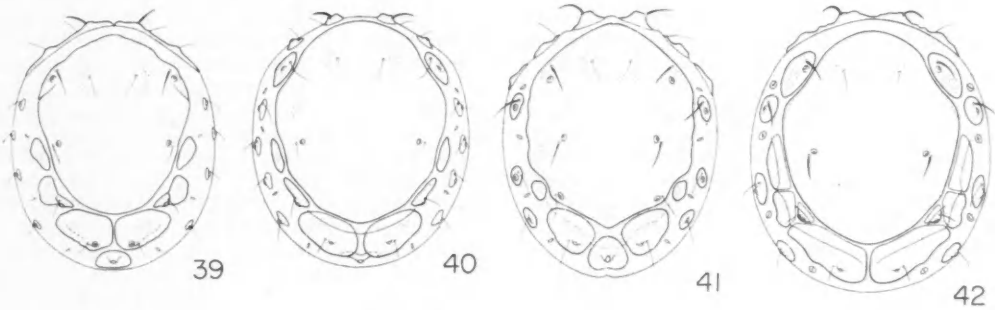

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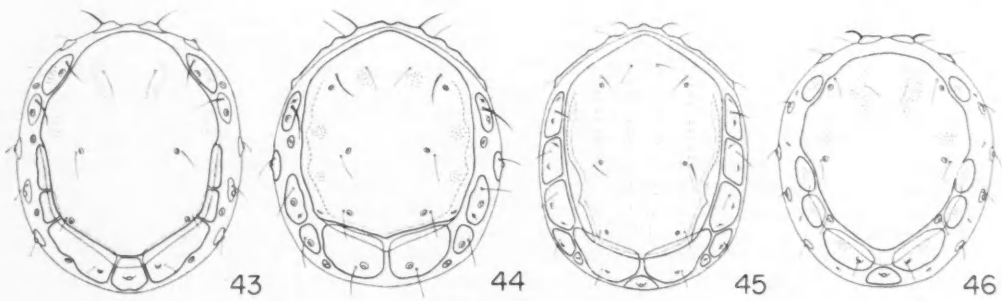

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[PLATE 3]

FIG. 33.—Schematic drawing of basic dorsal plates and glandularia in *Feltria*. FIGS. 34-46—Dorsal view of females. FIG. 34.—*Feltria multiscutata*, n. sp. FIG. 35.—*F. amoenella* Habeeb. FIG. 36.—*F. rubra nearctica*, n. ssp. FIG. 37.—*F. macroplata geometrica* Habeeb. FIG. 38.—*F. polyplacophora*, n. sp. FIG. 39.—*F. laversi*, n. sp. FIG. 40.—*F. rivophila* Habeeb. FIG. 41.—*F. flatheadensis*, n. sp. FIG. 42.—*F. minuta* Koenike. FIG. 43.—*F. lundbladi*, n. sp. FIG. 44.—*F. montanensis*, n. sp. FIG. 45.—*F. major*, n. sp. FIG. 46.—*F. purpurotincta* Habeeb.

closely related to *F. m. macroplata* of Japan and *F. m. geometrica* of North America. The multiscutate species do appear to belong to a species group separate from the uni-, bi-, or triscutate species of *Feltriella* but, in view of the great variation in degree of fusion of the dorsal plates within the groups, generic or subgeneric separation does not seem to be justified.

3. **Feltria (Feltriella) multiscutata,** new species

Figs. 1, 17, 34, 49, 60, 88

Male: Length 322μ-375μ; first and second coxae extending far posteriorly, with apodemes extending to middle of fourth coxae; posteromedial corners of fourth coxae bluntly pointed and reaching almost to genital field; first coxae separated medially; genital field 201μ in width; genital acetabula 17-18 on each side, these arranged mostly around periphery of acetabular plates (fig. 1); excretory pore fused with genital field; ventrolateral plates extending up onto the dorsum; dorsum with plates and glandularia arranged as follows: anteromedial plate relatively large and somewhat six-sided, length of this plate 150μ-176μ, width 92μ-116μ; dorsoglandularia D broadly fused medially; dorsal plates D fused medially; dorsal plates B and C, and dorsoglandularia B fused on their respective sides in the holotype specimen, but all separate on the paratype male; all other plates and glandularia separate and arranged as shown in figure 17; dorsal lengths of the palpal segments, P-I 17μ, P-II 58μ, P-III 24μ, P-IV 64μ, P-V 40μ; figure 88 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 72μ; III-Leg-5, 92μ; III-Leg-6, 94μ; figure 60 shows III-Leg-6.

Female: Length 366μ-610μ; first and second coxae extending far posteriorly, apodemes much shorter than in male; third and fourth coxae shaped much as in male (fig. 49); first coxae separated medially, the individual acetabular plates 76μ-132μ in width, 76μ-120μ in length; genital acetabula 13-39 on each side (this variation found within the same population); acetabula tending to be arranged about the periphery of the acetabular plates, in some specimens with two or three acetabula in the posteromedial quandrant of the plates, in others with several acetabula in this location; excretory pore extending up on the dorsum; dorsum typically with all plates and glandularia separate or with dorsal plates D fused medially (fig. 34); in two specimens the dorsoglandularia A fused with the anteromedial plate, length of this plate 140μ-179μ, width 124μ-152μ (in specimens with dorsoglandularia A fused to plate, width 176μ); dorsal lengths of the palpal segments, P-I 17μ-20μ, P-II 56μ-63μ, P-III 23μ-28μ, P-IV 60μ-76μ, P-V 44μ-50μ; P-V longer and comparatively thinner than in male; dorsal lengths of distal segments of first leg, I-Leg-4, 52μ-60μ; I-Leg-5, 57μ-70μ; I-Leg-6, 64μ-76μ; leg segments stocky.

Types: Holotype, adult male, collected in seepage-wetted mosses on the sheer face of a cliff along State Highway 5, in northwestern Lewis County, Washington, August 13, 1954; allotype female, same data; paratypes, 14 females, same data; 1 male, taken in a cold reocrene spring along the east shore of Flathead Lake, Lake County, Montana (T26N/R19W/-S29), June 28, 1954; 1 female, same data, on July 10, 1954.

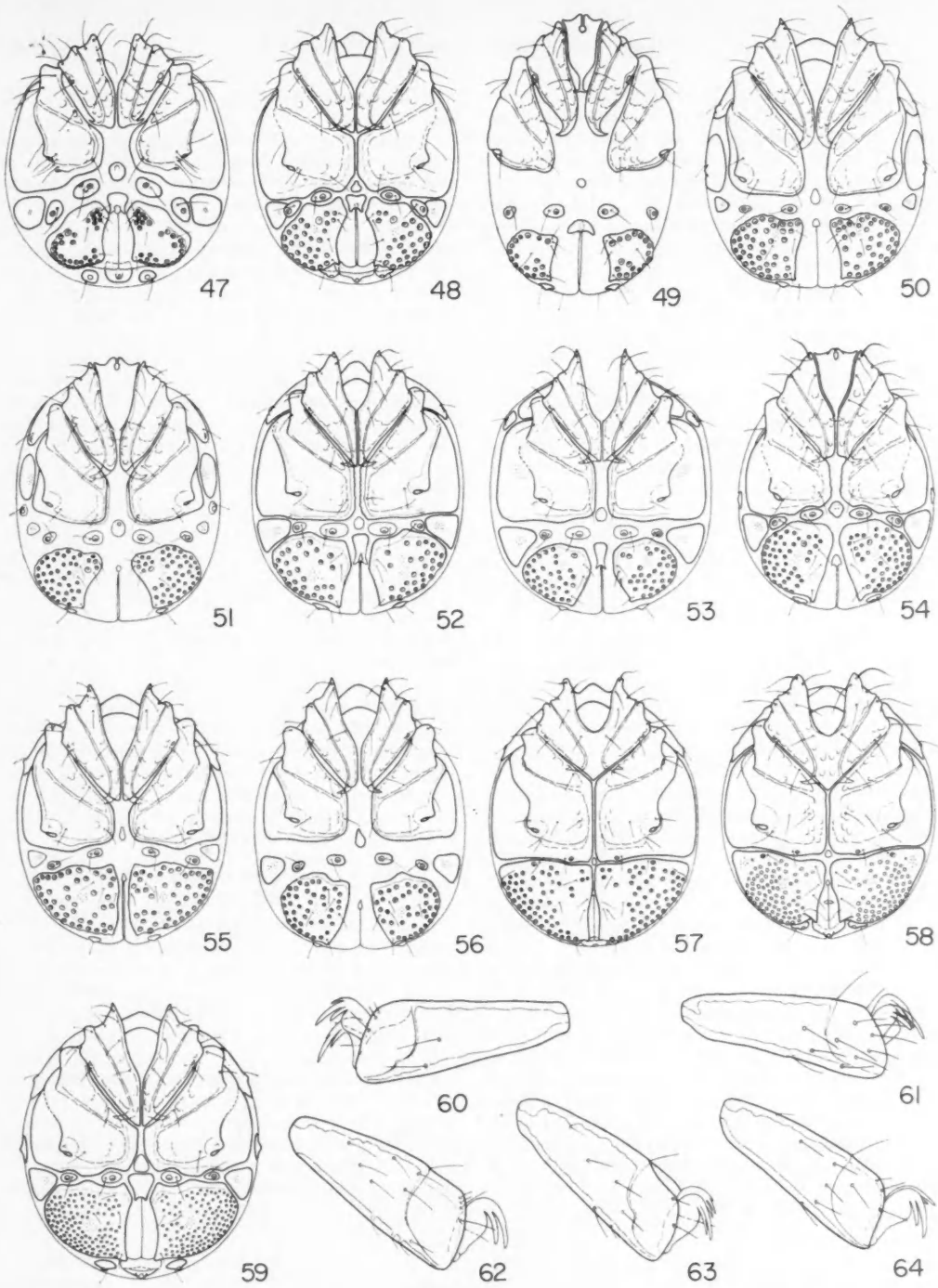
The structure of the coxae and genital field would seem to relate the present species to *F. menzeli* described by Walter (1922) from Switzerland. The male of *menzeli* differs in that the dorsum consists of an anterior plate made up of the fused anteromedial plate and dorsoglandularia A, and a posterior plate made up of the fused dorsal plates B,C,D,E and the dorsoglandularia B,C,D. The palp of *F. menzeli* is also slimmer and the genital field extends much farther laterally. The female of *menzeli* differs from *multiscutata* in that the anteromedial plate is separated into right and left halves. Actually, certain specimens of *multiscutata* give some indication of this separate condition in that the posterior end of the anteromedial plate is cleft and there are vague indications of a suture extending up the middle of the plate. The female of *F. menzeli* also has dorsal plates D and dorsoglandularia B fused medially into a single, large, median plate.

4. **Feltria (Feltriella) rubra nearctica,** new subspecies

Figs. 4, 21, 36, 51, 63, 89

Male: Length 331μ-350μ; coxal groups separated medially; median margin of third coxae approximately one-half length of fourth coxae; apodemes of first coxae extending to fourth coxae; both pairs of glandularia located between coxae and genital field free; genital field 176μ-198μ in width; genital opening small and located in center of acetabular plate; genital acetabula 35-42 on each side, one pair of acetabula much larger than others; anterior edge of acetabular plates with a pronounced median indentation (fig. 4); excretory pore fused with genital field; ventrolateral plates small and somewhat rounded; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A,B,C,D,E, and dorsoglandularia A,B,C; length of this plate 253μ-279μ, width 209μ-236μ; dorsoglandularia D relatively small and separate (fig. 21); all lateroglandularia separate; dorsal lengths of the palpal segments, P-I 16μ-20μ, P-II 68μ-76μ, P-III 28μ-30μ, P-IV 84μ-92μ, P-V 42μ-44μ; figure 89 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 64μ-72μ; III-Leg-5, 84μ-89μ; III-Leg-6, 96μ-103μ; figure 63 shows III-Leg-6.

Female: Length 332μ-393μ; coxal groups separated medially; apodemes of first coxae extending almost to fourth coxae (fig. 51); the individual acetabular plates 84μ-100μ in width, 74μ-90μ in length; genital acetabula 31-47 on each side, one pair of acetabula much larger than others; pregenital sclerite located between anteromedial portions of acetabular plates; ventrolateral plates very small and somewhat triangular in shape; dorsum with a large plate made up



[PLATE 4]

FIGS. 47-59.—Ventral view of females. FIG. 47.—*Feltria polyplacophora*, n. sp. FIG. 48.—*F. macroplata geometrica* Habeeb. FIG. 49.—*F. multiscutata*, n. sp. FIG. 50.—*F. amoenella* Habeeb. FIG. 51—*F. rubra nearctica*, n. ssp. FIG. 52.—*F. lundbladi*, n. sp. FIG. 53.—*F. purpurotincta* Habeeb. FIG. 54.—*F. flatheadensis*, n. sp. FIG. 55.—*F. rivophila* Habeeb. FIG. 56.—*F. laversi*, n. sp. FIG. 57.—*F. major*, n. sp. FIG. 58.—*F. montanensis*, n. sp. FIG. 59.—*F. minuta* Koenike.

FIGS. 60-64.—III-leg-6 of males. FIG. 60.—*Feltria multiscutata*, n. sp. FIG. 61.—*F. polyplacophora*, n. sp. FIG. 62.—*F. amoenella* Habeeb. FIG. 63.—*F. rubra nearctica*, n. sp. FIG. 64.—*F. macroplata geometrica* Habeeb.

of the fused anteromedial plate, dorsal plates B,D and dorsoglandularia A,B; length of this plate 240μ-279μ, width 192μ-223μ; other plates and glandularia separate as shown in figure 36; excretory pore plate large and dorsally located; dorsal lengths of the palpal segments, P-I 16μ-20μ, P-II 60μ-68μ, P-III 30μ-36μ, P-IV 82μ-92μ, P-V 48μ-51μ; dorsal lengths of the distal segments of the first leg, I-Leg-4, 56μ-62μ; I-Leg-5, 68μ-72μ; I-Leg-6, 76μ-80μ.

Types: Holotype, adult male, collected in a reocrene spring on the east side of Flathead Lake, Lake County, Montana (T24N/R19W/S22), July 1, 1954; allotype female, six paratype males, five paratype females, same data.

The new subspecies differs from the Old World form, *F. rubra rubra*, in that the male palp is longer and more slender, especially P-IV. The palp of *rubra rubra* more closely resembles that of the following species, *F. amoenella*, in proportions.

5. **Feltria (Feltriella) amoenella** Habeeb

Figs. 3, 20, 35, 50, 62, 75

F. *amoenella* Habeeb, 1955, Leaflets Acadian Biology No. 5: 1.
F. *amoenella* Habeeb, 1957, *Ibid.*, No. 16: 3.

Male: Length 314μ-349μ; coxal groups separated medially; median margin of third and fourth coxae of approximately same length; apodemes from first coxae of moderate length; both pairs of glandularia located between coxae and genital field free; genital field 168μ-204μ in width; genital opening small and located near center of acetabular plates; genital acetabula 44-55 on each side, typically with one pair of acetabula slightly larger than others; anterior edge of acetabular plates with a slight median indentation (fig. 3); excretory pore fused with genital field; ventrolateral plates very small and somewhat triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A,B,C,D,E, dorsoglandularia A,B,C,D, and lateroglandularia A; in some specimens dorsoglandularia D fused without a trace of a suture line, in other specimens with a slight suggestion of a suture anteromedially (fig. 20), and in still other specimens there is a strongly developed suture line with indentations laterally; dorsal plate having the apperance of hammered metal; dorsal lengths of the palpal segments, P-I 18μ-20μ, P-II 60μ-70μ, P-III 26μ-32μ, P-IV 70μ-80μ, P-V 38μ-42μ; figure 75 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 68μ-70μ; III-Leg-5, 84μ-90μ; III-Leg-6, 94μ-100μ; figure 62 illustrates III-Leg-6.

Female: Length 358μ-384μ; coxal groups separated medially; apodemes from first coxae of moderate length; the individual acetabular plates 88μ-104μ in width, 76μ-88μ in length; genital acetabula 39-52 on each side; pregenital sclerite small and located between anteromedial parts of acetabular plates; ventrolateral plates small and triangular in shape (fig. 50); dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A,B,C,D, and dorsoglandularia A,B; other plates and glandularia free; dorsoglandularia D relatively small (fig. 35); excretory pore moderate in size and dorsally located; dorsal lengths of the palpal segments, P-I 18μ-20μ, P-II 52μ-64μ, P-III 24μ-29μ, P-IV 64μ-77μ, P-V 42μ-45μ; dorsal lengths of the distal segments of the first leg, I-Leg-4, 56μ-60μ; I-Leg-5, 66μ-72μ; I-Leg-6, 76μ-86μ.

The type locality of this species was a waterfall associated with a brook near Mt. Albert, New Brunswick. It has since been taken as far south as North Carolina. The present author has collected *amoenella* in the Miner River, at the top of Miner's Falls, Alger County, and in a waterfall associated with Jacob's Creek, Keweenaw County, both localities in the Upper Peninsula of Michigan.

Feltria amoenella is most closely related to *F. rubra*, but differs in both sexes in having the dorsal plate resemble hammered metal. The male of *amoenella* also differs in having dorsoglandularia D and lateroglandularia A fused with the main dorsal shield. The female of the present species differs from the female of *rubra* in having dorsal plates A and C fused with the main dorsal shield.

6. **Feltria** (s. s.) **montanensis,** new species

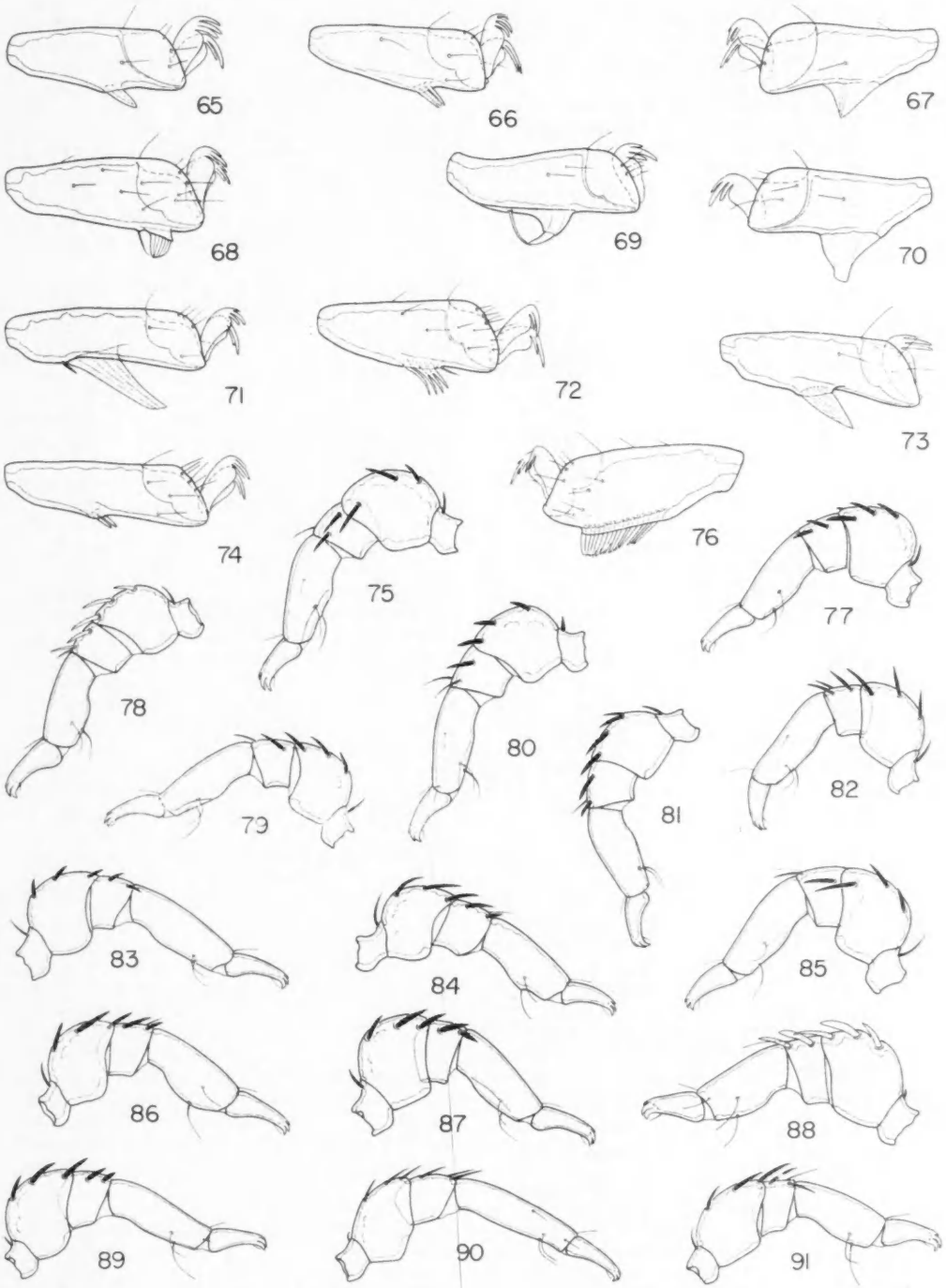
Figs. 6, 22, 44, 58, 72, 86

Male: Length 358μ-393μ, width 262μ-297μ; coxal groups and genital field fused into a ventral shield (fig. 6); apodemes from first coxae not extending to fourth coxae; with a ridge extending anterolaterally from the anterior portion of the fourth coxae; genital field 200μ-232μ in width; genital opening small and located near anterior edge of genital field; genital acetabula numerous; excretory pore fused with genital field, ventrolateral plates incorporated into the ventral shield; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A,B,C,D,E, and dorsoglandularia A,B,C; this plate somewhat bluntly pointed anteriorly and with a raised ridge

[PLATE 5]

FIGS. 65-76.—III-leg-6 of males. FIG. 65.—*Feltria parva*, n. sp. FIG. 66.—*F. flatheadensis*, n. sp. FIG. 67.—*F. oregonensis*, n. sp. FIG. 68.—*F. minuta* Koenike. FIG. 69.—*F. curviseta*, n. sp. FIG. 70.—*F. purpurotincta* Habeeb. FIG. 71.—*F. lundbladi*, n. sp. FIG. 72.—*F. montanensis*, n. sp. FIG. 73.—*F. rivophila* Habeeb. FIG. 74.—*F. laversi*, n. sp. FIG. 75.—*F. amoenella* Habeeb. FIG. 76.—*F. major*, n. sp.

FIGS. 77-91.—Palp of males. FIG. 77.—Feltria *laversi*, n. sp. FIG. 78.— *F. parva*, n. sp. FIG. 79.—*F. flatheadensis*, n. sp. FIG. 80.—*F. minuta* Koenike. FIG. 81.—*F. lundbladi*, n. sp. FIG. 82.—*F. curviseta*, n. sp. FIG. 83.—*F. purpurotincta* Habeeb. FIG. 84.—*F. major*, n. sp. FIG. 85.—*F. oregonensis*, n. sp. FIG. 86.—*F. montanensis*, n. sp. FIG. 87.—*F. rivophila* Habeeb. FIG. 88.—*F. multiscutata*, n. sp. FIG. 89.—*F. rubra nearctica*, n. ssp. FIG. 90.—*F. macroplata geometrica* Habeeb. FIG. 91.—*F. polyplacophora*, n. sp.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closely paralleling the lateral edges; length of this plate 279μ-318μ, width 218μ-244μ; dorsoglandularia D separate and somewhat rectangular in shape; lateroglandularia enlarged but separate (fig. 22); dorsal lengths of the palpal segments, P-I 16μ-20μ, P-II 60μ-64μ, P-III 32μ-35μ, P-IV 72μ-78μ, P-V 44μ-47μ; figure 86 shows the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 76μ-80μ; III-Leg-5, 88μ-90μ; III-Leg-6, 92μ-99μ; ventral side of III-Leg-6 with 4-6 enlarged setae and a single, smaller, more distally placed seta (fig. 72); the larger setae separate and sharp pointed at distal end but may or may not be fused near their bases.

Female: Length 384μ-454μ, width 314μ-375μ; anterior coxal groups fused medially, posterior coxal groups free (fig. 58); apodemes from first coxae very short; the individual acetabular plates (including the fused ventrolateral plates) 136μ-168μ in width, 92μ-112μ in length; genital acetabula numerous; lateral pair of glandularia between coxae and genital field fused with acetabular plates, medial pair of glandularia fused with fourth coxae; excretory pore ventral, fused with the postgenital sclerite, pregenital sclerite small, not extending to anterior portions of acetabular plates; fusion of dorsal plates and glandularia similar to that of male; large plate less pointed anteriorly than in male (fig. 44); length of this plate 288μ-349μ, width 244μ-296μ; dorsal lengths of the palpal segments, P-I 18μ-22μ, P-II 52μ-60μ, P-III 30μ-35μ, P-IV 70μ-80μ, P-V 46μ-52μ; dorsal lengths of the distal segments of the first leg, I-Leg-4, 56μ-63μ; I-Leg-5, 66μ-76μ; I-Leg-6, 76μ-83μ.

Types: Holotype, adult male, taken in Johnson Creek on the road between Big Fork and Swan Lake, Lake County, Montana (T26N/R19W/S14), June 30, 1954; allotype female, same data; paratypes, 11 males, 6 females, same data; 4 males, 4 females, collected in a stream in Deer Lodge National Forest, Dear Lodge County, Montana, July 3, 1954; 1 male, 1 female, from Potter Creek near Rodger's Lake, Flathead County, Montana (T27N/R23W/S21), July 16, 1954; 1 male, 5 females, taken in Chamas Creek, Glacier National Park, July 21, 1959; 1 female, collected in a small stream near Tioga Lake, Mono County, California, August 4, 1954.

The present species seems most closely related to *F. ezoensis* described by Imamura (1954) from Hokkaido, Japan. *F. ezoensis*, known only from the female, differs from *montanensis* in that the third and fourth coxae are fused medially, the anterior end possesses a well-developed projection, and there are fewer genital acetabula.

7. **Feltria** (s. s.) **major,** new species

Figs. 7, 23, 45, 57, 76, 84

Male: Length 487μ, width 331μ; coxal groups and genital field fused into a ventral shield (fig. 7); apodemes from first coxae very short; a very pronounced ridge extending anterolaterally from the anterior portion of the fourth coxae; genital field 300μ in width; genital opening small and located near anterior edge of genital field; genital acetabula numerous; excretory pore fused with the genital field; ventrolateral plates incorporated into the ventral shield; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A,B,C,D,E, and the dorsoglandularia A,B,C; this plate bluntly pointed at anterior end and with a very pronounced ridge on both sides near lateral margins, this ridge abruptly curving medially in region opposite posterior end of lateroglandularia B; smaller, more medially located ridges present on dorsal plate as indicated by dotted lines in figure 23; length of this plate 384μ, width 271μ; dorsoglandularia D separate; lateroglandularia enlarged but separate; dorsal lengths of the palpal segments, P-I 22μ, P-II 62μ, P-III 36μ, P-IV 76μ, P-V 42μ; figure 84 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 92μ; III-Leg-5, 92μ; III-Leg-6, 110μ; ventral side of III-Leg-6 with 15 enlarged, closely-set setae; these setae tend to be lightly fused along most of their length (fig. 76).

Female: Length 524μ, width 402μ; anterior coxal groups fused medially, posterior coxal groups free (fig. 57); apodemes from first coxae very short; width of individual acetabular plates (including the fused ventrolateral plate) 176μ, length 156μ; genital acetabula numerous; lateral pair of glandularia between coxae and genital field fused with acetabular plates, medial pair of glandularia fused with fourth coxae; excretory pore terminal and extending between the posterior corners of dorsoglandularia D; pregenital sclerite small, not extending to anterior corners of acetabular plates; fusion of dorsal plates and glandularia similar to that of male; large plate less pointed anteriorly than in male (fig. 45), length of this plate 419μ, width 288μ; dorsal lengths of the palpal segments, P-I 26μ, P-II 62μ, P-III 34μ, P-IV 72μ, P-V 44μ; first leg oriented on slide in such a way that measurements of length cannot be obtained.

Types: Holotype, adult male, collected in a stream in Deer Lodge National Forest (Spring Hill Picnic Area), Deer Lodge County, Montana, July 3, 1954; allotype female, same data.

Feltria major is related to *F. montanensis* but may be easily separated as follows: the male of *major* has a much greater number of setae on III-Leg-6 and the genital field is much wider and proportionally longer. The female of the present species differs in the shape of the dorsal plates (compare figs. 44 and 45). The female of *F. major* also closely resembles the female of *F. ezoensis* Imamura but differs in having the third and fourth coxae separated medially.

8. **Feltria** (s. s.) **lundbladi,** new species

Figs. 5, 24, 43, 52, 71, 81

Male: Length 336μ, width 262μ; coxal groups and genital field fused into a ventral shield (fig. 5); apodemes from first coxae extending to fourth coxae; genital field 188μ in width; genital opening small and

located near anterior edge of genital field; genital acetabula 29-34 on each side; excretory pore fused with the genital field; ventrolateral plates incorporated into the ventral shield but their position indicated by suture lines; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A,B,C,D,E, and the dorsoglandularia A,B,C (fig. 24); length of this plate 279μ, width 209μ; dorsoglandularia D separate, each 100μ-103μ in width; lateroglandularia separate and slightly enlarged; dorsal lengths of the palpal segments, P-I 16μ, P-II 56μ, P-III 30μ, P-IV 68μ, P-V 38μ; figure 81 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 68μ; III-Leg-5, 72μ; III-Leg-6, 88μ; ventral side of III-Leg-6 with 5-6 long, fused setae, and a single, short, proximally located seta (fig. 71); length of fused setae approximately 44μ.

Female: Length 401μ-419μ, width 331μ-349μ; coxal groups separate; apodemes of first coxae short; width of individual acetabular plates 124μ-136μ, length 99μ-104μ; genital acetabula 28-30 on each side; both pairs of glandularia between coxae and genital field free; a rounded anterior projection of the acetabular plates extending between the glandularia of each side (fig. 52); pregenital sclerite long and narrow, extending anterior to the acetabular plates; ventrolateral plates free and of moderate size; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates B,D, and dorsoglandularia B,C; length of this plate 335μ-340μ, with 269μ-271μ; dorsal plates A fused with dorsoglandularia A on their respective sides to form small oval plates flanking the anterolateral edges of the large plate; dorsal plates C,E, forming small, somewhat rectangular plates which are free, but closely appressed to the large plate (fig. 43); dorsoglandularia D free and widely separated medially by the excretory pore plate; excretory pore plate somewhat five-angled and extending to the large dorsal plate; lateroglandularia free, lateroglandularia A of moderate size, others smaller; dorsal lengths of the palpal segments, P-I 17μ-18μ, P-II 50μ-52μ, P-III 30μ-32μ, P-IV 63μ-66μ, P-V 40μ; dorsal lengths of the distal segments of the first leg, I-Leg-4, 54μ-58μ; I-Leg-5, 62μ-65μ; I-Leg-6, 70μ-76μ.

Types: Holotype, adult male, taken in Johnson Creek on road between Big Fork and Swan Lake, Lake County, Montana (T26N/R19W/S14), June 30, 1954; allotype female, one paratype female, same data.

Males of *F. lundbladi* may be separated from all other species of *Feltria* by the following combination of characters: coxae and genital field fused into a ventral shield; dorsoglandularia D separate; III-Leg-6 with 5-6 very long setae fused into a blade-like projection.

9. **Feltria,** (s. s.) **purpurotincta** Habeeb

Figs. 9, 25, 46, 53, 70, 83

F. purpurotincta Habeeb, 1955, Leaflets Acadian Biology No. 5: 3.

Male: Length 401μ, width 314μ; coxal groups separate (but see remarks under this species); median margin of fourth coxae much reduced; apodemes from first coxae short; lateral pair of glandularia between coxae and genital field free, median pair fused with the fourth coxae; genital field 201μ in width, 140μ in length; genital acetabula 34-35 on each side; genital opening located in anterior projection of the genital field; genital field with median suture line (fig. 9); excretory pore fused with the genital field; ventrolateral plates large, and somewhat triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, C, D, E, and dorsoglandularia A, B, C, D; dorsal plate with ridges along posterolateral edges as indicated by dotted lines in figure 25; length of this plate 341μ, width 244μ; lateroglandularia small; dorsal lengths of the palpal segments, P-I 20μ, P-II 64μ, P-III 36μ, P-IV 90μ, P-V 44μ; chaetotaxy of palp illustrated by figure 83; dorsal lengths of the distal segments of the third leg, III-Leg-4, 70μ; III-Leg-5, 84μ; III-Leg-6, 88μ; ventral side of III-Leg-6 with setae modified into a triangular structure as indicated in figure 70, fusion of setae so complete that it is difficult to see line of demarcation between them.

Female: Length 419μ, width 349μ; coxal groups separate; apodemes from first coxae short; width of individual acetabular plates 103μ-105μ, length 92μ-95μ; genital acetabula 32-34 on each side; both pairs of glandularia between coxae and genital field free, acetabular plates not extending between these glandularia; pregenital sclerite long and relatively narrow (fig. 53); ventrolateral plates large and somewhat triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates B, D, and dorsoglandularia A, B; length of this plate 306μ, width 271μ; dorsal plates A, C, E small, somewhat oval and lying close to the large plate; in the single female specimen studied, dorsal plate C lightly fused with the large plate on the right side (fig. 46); dorsoglandularia C very small and separated from the large plate; lateroglandularia small and free; excretory pore plate dorsal, somewhat triangular in shape; dorsal lengths of the palpal segments, P-I 22μ, P-II 62μ, P-III 36μ, P-IV 84μ, P-V 44μ; first legs oriented on slide in such a manner that measurements of length cannot be obtained.

Two specimens, a male and female, were collected in Potter Creek near Rodger's Lake, Flathead County, Montana (T27N/R23W/S21), July 16, 1954. Habeeb (1955a) described specimens from New Jersey and New Brunswick with the third and fourth coxae fused medially as *F. purpurotincta*. Individuals from New Jersey in which the third and fourth coxae were separated medially were described as a subspecies, *F. purpurotincta separatella*. Habeeb stated that *separatella* was also smaller and possessed fewer acetabula. However, the western specimens have the third and fourth coxae separated medially but are larger and possess more acetabula than *purpurotincta*. It is known from other species of *Feltria*

(see description of *F. macroplata geometrica*) that the coxal groups may be either separate or fused medially, even within the same population. It is a simple step for secondary sclerotization to fuse the coxal plates medially, especially when they are as close together as is shown in figure 9. It seems highly probable that *separatella* is only a "form" of *purpurotincta*, but until longer series are available, the true status of *separatella* is unknown.

10. **Feltria** (s. s.) **curviseta,** new species

Figs. 10, *26*, *69*, *82*

Male: Length 480μ-515μ, width 401μ-418μ; coxal groups separate medially; apodemes from first coxae extending to fourth coxae; both pairs of glandularia between coxae and genital field small but free; genital field 376μ in width; genital acetabula numerous; genital field with rounded projections anteriorly between the glandularia; genital opening small, located slightly anterior to the middle of the genital field; genital field with a deep, median identation at the posterior end; excretory pore lying free in the integument; ventrolateral plates very narrow and somewhat oval in shape (fig. 10); dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, C, D, E, and dorsoglandularia A, B, C, D; dorsal plate with a median indentation at posterior end (fig. *26*); length of this plate 419μ-454μ, width 345μ-367μ; lateroglandularia small and lying free in the integument; dorsal lengths of the palpal segments, P-I 24μ-28μ, P-II 84μ-91μ, P-III 40μ-44μ, P-IV 104μ-116μ, P-V 56μ-60μ; figure 82 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 92μ-95μ; III-Leg-5, 108μ-124μ; III-Leg-6, 104μ-110μ; ventral side of III-Leg-6 with a large thickened seta which curves proximally, and a thinner, more proximally located seta which curves distally; tips of these two setae touching (fig. *69*).

Female: Unknown.

Types: Holotype, adult male, collected in algae masses on rocks in the Little Blackfoot River, Powell County, Montana, July 7, 1954; one paratype male, taken in a small stream along the edge of Hungry Horse Dam, Flathead County, Montana, July 9, 1954.

Feltria curviseta may be easily separated from all known species of *Feltria* by the following combination of characters: large size; all dorsal plates and dorsoglandularia fused into a single plate; structure of the genital region with the unusually shaped ventrolateral plates and the excretory pore lying free in the integument; and especially the odd arrangement of the setae on the ventral side of III-Leg-6 (fig. *69*).

11. **Feltria** (s. s.) **minuta** Koenike

Figs. 15, *32*, *42*, *59*, *68*, *80*

F. *minuta* Koenike, 1892, Zool. Anz. 15: 323 (original description)
F. *minuta* Koenike, 1895, Abh. naturwiss. Ver. Bremen 13: 192.
F. *minuta* Bergstrom, 1953, Trans. Amer. Microsc. Soc. 72: 161.
F. *minuta* Habeeb, 1955, Leaflets Acadian Biology No. 5: 4.
F. *minuta* Habeeb, 1956, *Ibid.* No. 11: 2.
F. *minuta* Habeeb, 1957, Ibid No. 16: 3.

Male: Length 349μ-402μ, width 271μ-314μ; coxal groups separated medially; apodemes from first coxae extending to fourth coxae; both pairs of glandularia between coxae and genital field very small and lying free in the integument; genital field 224μ-260μ in width; genital acetabula numerous; genital field with very slight, rounded projections between the glandularia; genital opening small and located near anterior edge of genital field, often closer than shown in figure 16; excretory pore fused with genital field; ventrolateral plates of moderate size and triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, C, D, E, and dorsoglandularia A, B, C; dorsoglandularia D separated and directed somewhat anteromedially (fig. *32*); lateroglandularia small and unfused; dorsal lengths of the palpal segments, P-I 18μ-22μ, P-II 58μ-66μ, P-III 30μ-36μ, P-IV 74μ-82μ, P-V 40μ-44μ; figure 80 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 74μ-84μ; III-Leg-5, 86μ-95μ; III-Leg-6, 88μ-102μ; ventral side of III-Leg-6 with a group of 5-6 fused, short, enlarged setae located near the distal end; usually with a single, thin seta placed slightly proximal to the enlarged setae (fig. 68), but this seta absent in some individuals.

Female: Length 376μ-463μ, width 341μ-384μ; coxal groups separate; apodemes from first coxae very short; width of individual acetabular plates 124μ-148μ, length 104μ-132μ; genital acetabula numerous; both pairs of glandularia between the coxae and the genital field free; anterior edge of acetabular plates with rounded projections extending between these glandularia (fig. 59); pregenital sclerite somewhat four-angled; ventrolateral plates moderate in size and triangular in shape; excretory pore terminal; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates B, D, and dorsoglandularia B; length of the plate 306μ-366μ, width 253μ-288μ; dorsal plates A and dorsoglandularia A fused on their respective sides into oval plates along the anterolateral margin of the large plate; dorsal plates C, E separate but closely appressed to the lateral margin of the large plate; dorsoglandularia C small, lying near posterolateral margin of large plate; dorsoglandularia D separate and directed somewhat anterolaterally (fig. 42); dorsal lengths of the palpal segments, P-I 20μ-23μ, P-II 50μ-58μ, P-III 28μ-34μ, P-IV 68μ-78μ, P-V 39μ-44μ; dorsal lengths of the distal segments of the first leg, I-Leg-4, 54μ-60μ; I-Leg-5, 62μ-72μ; I-Leg-6, 76μ-83μ.

This holarctic species has been collected in widely scattered localities in Europe and Asia. In eastern North America, *F. minuta* has been reported from New Brunswick, New Jersey, and Georgia. Within

the range covered by this paper, *F. minuta* has been taken in the Sturgeon River, Cheboygan County, Michigan; Miner River, Alger County, Michigan; a small creek in Teton County, Wyoming; Chamas Creek in Glacier National Park; a small stream near Tioga Lake, Mono County, California; Pecos River in San Miguel County, New Mexico; Bergstrom (1953) reported this species from Santa Fe and Sandoval Counties, New Mexico.

12. **Feltria** (s. s.) **oregonensis,** new species

Figs. 12, 27, 67, 85

Male: Length 349μ, width 253μ; first and second coxae fused medially, third and fourth coxae slightly separated medially; apodemes from first coxae extending to fourth coxae; median margin of third and fourth coxae of approximately same length; median pair of glandularia located between coxae and genital field fused with the fourth coxae, lateral pair of glandularia free (right glandularium missing); genital field 188μ in width; genital opening moderately large and located near middle of genital field; genital acetabula 37-40 on each side; excretory pore fused with the genital field; ventrolateral plates of moderate size and triangular in shape (fig. 12); dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, C, D, E, and dorsoglandularia A, B, C; length of this plate 258μ, width 209μ; dorsoglandularia D and the lateroglandularia separate (fig. 27); dorsal lengths of the palpal segments, P-I 20μ, P-II 64μ, P-III 32μ, P-IV 78μ, P-V 41μ; figure 85 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 68μ; III-Leg-5, 92μ; III-Leg-6, 88μ; ventral side of III-Leg-6 with enlarged setae fused into a triangular structure as illustrated in figure 67; fusion of these setae almost complete, sutures indicating the individual setae almost obliterated.

Female: Unknown.

Types: Holotype male, collected in the south fork of Iron Creek along State Highway No. 35, Hood River County, Oregon, August 11, 1954.

Feltria oregonensis may be separated from other known species of *Feltria* by the following combination of characters: acetabular plates with a large, centrally located genital opening; acetabular plates not fused with the coxae; setae on the ventral side of III-Leg-6 fused into a triangular structure as illustrated in figure 67.

13. **Feltria** (s. s.) **flatheadensis,** new species

Figs. 11, 28, 54, 66, 79

Male: Length 358μ, width 253μ; coxal groups separate; apodemes from first coxae short; both pairs of glandularia between coxae and genital field free; genital field 184μ in width; anterior edge of genital field with a median indentation (fig. 11); genital opening small, located closer to anterior edge of acetabular plates; genital acetabula 38-39 on each side; excretory pore fused with the genital field; ventrolateral plates medium-sized and somewhat triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, C, D, and dorsoglandularia A, B, C; length of this plate 262μ, width 210μ; dorsal plates E small and fused, located along the posterolateral edges of the large plate (fig. 28); dorsoglandularia D separate and of moderate size; lateroglandularia small and lying free in the integument; dorsal lengths of the palpal segments, P-I 18μ, P-II 56μ, P-III 32μ, P-IV 76μ, P-V 43μ; figure 79 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 68μ; III-Leg-5, 80μ; III-Leg-6, 94μ; ventral side of III-Leg-6 with three, distally-pointing setae of moderate length, these slightly, if at all, fused medially (fig. 66).

Female: Length 401μ-419μ, width 306μ-314μ; coxal groups separate; apodemes from first coxae short; width of individual acetabular plates 104μ-109μ, length 104μ-114μ; genital acetabula 40-49 on each side; acetabular plates extending anteriorly far beyond the pregenital sclerite (fig. 54); both pairs of glandularia between coxae and genital field separate; ventrolateral plates of moderate size and somewhat triangular in shape; fusion of the dorsal plates and glandularia similar to that of male (fig. 41); length of the large dorsal plate 296μ-305μ, width 230μ-244μ; excretory pore plate dorsal, somewhat triangular in shape and extending between dorsoglandularia D; dorsal lengths of the palpal segments, P-I 18μ, P-II 56μ-60μ, P-III 28μ-31μ, P-IV 76μ, P-V 44μ-46μ; dorsal lengths of the distal segments of the first leg, I-Leg-4, 56μ; I-Leg-5, 62μ-66μ; I-Leg-6, 78μ-80μ.

Types: Holotype, adult male, taken in a reocrene spring along the east side of Flathead Lake, Lake County, Montana (T24N/R19W/S22), July 1, 1954; allotype female, 13 paratype females, same data.

Males of the present species may be separated from all other known species of *Feltria* by the following combination of characters: dorsum with the dorsal plates E separate from the large plate; acetabular plates with a median indentation on anterior edge. The females of *flatheadensis* are distinguished by their possession of a dorsum with plate and glandularia fusion similar to male, and acetabular plates which extend anteriorly far beyond the pregenital sclerite.

14. **Feltria** (s. s.) **parva,** new species

Figs. 13, 29, 65, 78

Male: Length 228μ-314μ, width 164μ-244μ; first and second coxae touching but not fused medially; third and fourth coxae separated medially; median margins of third and fourth coxae of approximately same length; apodemes from first coxae extending to middle of fourth coxae; both pairs of glandularia between coxae and genital field lying free in the integument; genital field 104μ-176μ in width; genital acetabula 17-35 on each side; genital opening located near anterior edge of genital field; genital field with a rounded, median projection; this projection not extending between fourth coxae; genital field

with a median suture line (fig. 13); excretory pore fused with the genital field; ventrolateral plates of moderate size and triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, C, D, E, and dorsoglandularia A, B, C; dorsoglandularia D small and separate (fig. 29); lateroglandularia small and separate; dorsal lengths of the palpal segments, P-I 12μ-16μ, P-II 40μ-56μ, P-III 18μ-32μ, P-IV 48μ-74μ, P-V 32μ-42μ; figure 78 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 44μ-64μ; III-Leg-5, 50μ-72μ; III-Leg-6, 64μ-92μ; ventral side of III-Leg-6 typically with three, occasionally two, fused, rather short setae as shown in figure 65; in two specimens these setae were fused near bases but distal ends were free.

Female: Not known with certainty.

Types: Holotype, adult male, collected in the Rogue River, 1 mile south of Trail, Jackson County, Oregon, August 10, 1954; paratypes: one male, same data as holotype; one male, taken in the Smith River, 2 miles southwest of Gasquet, Del Norte County, California, August 10, 1954; one male, Pecos River, 2 miles north of Pecos, San Miguel County, New Mexico, August 2, 1954; one male, taken in a reocrene spring along the east side of Flathead Lake, Lake County, Montana (T24N/R19W/S22), July 1, 1954.

Feltria parva seems most closely related to the following two species, *F. rivophila* Habeeb and *F. laversi* n. sp., but differs as follows: anterior edge of genital field not extending between fourth coxae; median pair of glandularia between coxae and genital field free rather than fused with the fourth coxae; dorsoglandularia D separate rather than fused medially. There seems to be an extremely great variation in body size and number of genital acetabula. The larger measurements belong to the specimen from Montana. The smallest measurements belong to one of the individuals from Oregon. However, the holotype, also from the Oregon collection, is closer to the Montana specimen, as far as measurements are concerned, than to the other individual from its own population. This strongly suggests that, in spite of the great size variation, all of the above described specimens are members of a single species. The measurements of the holotype closely approach those of the specimens from California and New Mexico.

15. **Feltria** (s. s.) **rivophila** Habeeb

Figs. 14, 30, 40, 55, 73, 87

F. rivophila Habeeb, 1955, Leaflets Acadian Biology No. 5: 2.

Male: Length 332μ-353μ, width 244μ-279μ; coxal groups separated medially, second and third coxae fused on their respective sides; median margin of fourth coxae greatly reduced; apodemes from first coxae extending to middle of fourth coxae; median pair of glandularia between coxae and genital field fused with the fourth coxae (fig. 14), lateral pair of glandularia free; genital field 202μ-236μ in width, 116μ-124μ in length; genital field extending between the posterior portions of the fourth coxae; genital opening small, located near anterior edge of genital field; genital acetabula 42-54 on each side; excretory pore fused with the genital field; ventrolateral plates large and triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, C, D, E, and dorsoglandularia A, B, C; length of this plate 244μ-261μ, width 213μ-244μ; dorsoglandularia D fused medially (fig. 30); lateroglandularia lying free in the integument; dorsal lengths of the palpal segments, P-I 18μ-22μ, P-II 58μ-68μ, P-III 28μ-32μ, P-IV 64μ-70μ, P-V 42μ-44μ; figure 87 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 68μ-76μ; III-Leg-5, 78μ-84μ; III-Leg-6, 88μ-99μ; ventral side of III-Leg-6 with 5-8 medially fused setae of moderate length; these setae may be fused to the very tip and tapering to a point as shown in figure 73, or with tips of setae unfused and not tapering to a point (i.e., tip truncate).

Female: Length 384μ-462μ, width 305μ-366μ; coxal groups separate; apodemes from first coxae short; width of individual acetabular plates 120μ-144μ, length 100μ-107μ; genital acetabula 41-57 on each side; pregenital sclerite very small, not extending anteriorly as far as anterior edges of acetabular plates (fig. 55); both pairs of glandularia between coxae and the genital field lying free in the integument; ventrolateral plates small and triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates B, D, and dorsoglandularia B; length of this plate 279μ-306μ, width 235μ-270μ; dorsal plates A and dorsoglandularia A fused into oval plates flanking the anterolateral margin of the large plate; dorsal plate C lying free; dorsal plates E fused with the dorsoglandularia C, these lying close to the posterolateral margin of the large plate (the smaller plates foreshortened in figure 40; if the specimen had been flattened during slide making, they would appear more like the homologous structures in figure 39); dorsoglandularia D separate, excretory pore not extending between them; dorsal lengths of the palpal segments, P-I 18μ-21μ, P-II 58μ-64μ, P-III 32μ-40μ, P-IV 72μ-78μ, P-V 48μ-50μ; dorsal lengths of the distal segments of the first leg, I-Leg-4, 58μ-61μ; I-Leg-5, 72μ-76μ; I-Leg-6, 82μ-90μ.

Feltria rivophila was described by Habeeb (1955a) from specimens collected in New Brunswick. The present author has taken this species from cold streams in Barry, Lake, and Wexford Counties in the Lower Peninsula and Alger County in the Upper Peninsula of Michigan. *F. rivophila* is most closely related to the following species, *F. laversi* n. sp. (see remarks under the latter).

16. **Feltria** (s. s.) **laversi,** new species

Figs. 16, 31, 39, 56, 74, 77

Male: Length 296μ-358μ, width 244μ-288μ; coxal groups separated medially, second and third coxae

fused on their respective sides; median margin of the fourth coxae greatly reduced; apodemes from first coxae extending to middle of the fourth coxae; median pair of glandularia between coxae and the genital field fused with the fourth coxae, lateral pair of glandularia free; genital field 164μ-196μ in width, 84μ-114μ in length; genital field extending between the posterior portions of the fourth coxae (fig. 16); genital opening small, located near anterior edge of genital field; genital acetabula 27-54 on each side; excretory pore lying free in the integument; ventrolateral plates large and triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, C, D, E, and dorsoglandularia A, B, C; length of this plate 231μ-270μ, width 209μ-244μ; dorsoglandularia D fused medially (fig. 31); lateroglandularia lying free in the integument; dorsal lengths of the palpal segments, P-I 14μ-18μ, P-II 56μ-64μ, P-III 27μ-32μ, P-IV 60μ-68μ, P-V 40μ-43μ; figure 77 illustrates the chaetotaxy of the palp; dorsal lengths of the distal segments of the third leg, III-Leg-4, 66μ-72μ; III-Leg-5, 78μ-84μ; III-Leg-6, 88μ-98μ; ventral side of III-Leg-6 with one to three heavy setae of moderate length, these may be separated medially as shown in figure 74 or fused to a degree which makes it difficult to discern lines of demarcation between the individual setae.

Female: Length 393μ-436μ, width 296μ-349μ; coxal groups separated; apodemes from first coxae short; width of individual acetabular plates 99μ-112μ, length 92μ-108μ; genital acetabula 35-51 on each side; pregenital sclerite very small, not extending as far as anterior edges of acetabular plates; both pairs of glandularia between the coxae and the genital field lying free in the integument (fig. 56); ventrolateral plates of medium size and somewhat triangular in shape; dorsum with a large plate made up of the fused anteromedial plate, dorsal plates A, B, D, and dorsoglandularia A, B, although fusion of dorsal plates A and dorsoglandularia A not complete posteriorly (fig. 39); length of large dorsal plate 248μ-306μ, width 210μ-253μ; dorsal plates C lying free; dorsal plates E lightly fused with dorsoglandularia C, and lying near posterolateral margin of large plate; dorsoglandularia D separate, excretory pore not extending between them; dorsal lengths of the palpal segments, P-I, 18μ-24μ, P-II 56μ-64μ, P-III 38μ-44μ, P-IV 72μ-87μ, P-V 48μ-56μ; dorsal lengths of the distal segments of the first leg, I-Leg-4, 56μ-64μ; I-Leg-5, 65μ-76μ; I-Leg-6, 84μ-92μ.

Types: Holotype, adult male, collected in Johnson Creek on road between Big Fork and Swan Lake, Lake County, Montana (T26N/R19W/S14), June 30, 1954; allotype, adult female, same data; paratypes, six males, two females, same data as holotype; one male, two females, taken in a small creek (tributary of the Snake River) between Yellowstone and Grand Teton National Parks, Teton County, Wyoming, July 5, 1954.

Feltria laversi is most closely related to the preceding species, *F. rivophila*, but differs as follows: the male of *laversi* possesses a narrower genital field and the excretory pore lies free in the integument; III-Leg-6 in the male of *laversi* has one to three short setae (fig. 74) in contrast to five to eight longer setae in the male of *rivophila* (fig. 73). In the females, the genital field of *F. laversi* is not as wide, and is rounded laterally (compare figures 55 and 56).

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Frass Studies of the Armyworm, *Pseudaletia unipuncta*[1]

D. D. POND[2]

Horticultural Crops Section, Research Station,
Canada Department of Agriculture, Fredericton, N. B.

ABSTRACT

Experiments conducted from 1956 to 1959 showed: (1) Frass pellets of the larvae have characteristic shapes and sizes which can be used as accurately as head-capsule measurements for determining the various instars; (2) Frass-pellet measurements in successive instars fit approximately to a geometric progression with an average ratio of 1.8; (3) Frass contains water-soluble substances and, when exposed to rainfall, may lose from 2% to 35% of its weight, depending upon the instar, though its volume is not affected; (4) At 62° F. the duration of the larval life span was almost double that at 72°, but the number of frass pellets produced increased only slightly; the total weight of frass was higher at 62° and the pupal weights were lower than for individuals reared at 72°; and (5) Temperature was the most important of the factors affecting frass yield.

See page 134 for footnotes 1 and 2.

Though the armyworm, *Pseudaletia* (*Cirphus*) *unipuncta* (Haw.), is so commonly destructive, very little is known of the causes of its outbreaks. That environmental factors hold the key for them has been suspected for some time. The outbreaks of 1896 and 1914 were treated in detail by Panton (1897) and by Gibson (1915), respectively, who reported certain larval habits, with descriptions, and dealt with parasites, predators, and the control measures adopted. There is a definite need, however, for a reliable method of determining trends of larval activity and the causes underlying the sudden outbreaks, and also the reasons for their abrupt termination.

The only direct method of obtaining such information is to examine a number of fields to determine the prevalence of the insects. This provides useful information on the age of the larvae and their number per square foot, but does not give any indication of the possibility that an outbreak may occur. Like all field sampling, this method is laborious and time consuming, and is subject to large variations depending on the instars that are present at the time of sampling. The first three instars are very difficult to observe in the field, and may easily be overlooked. Because of the many difficulties involved in direct sampling, it seemed logical, on undertaking these studies, to consider other methods.

Such methods were suggested by the work of Morris (1949), Thomson (1949), and others who have used frass drop or larval excrement as a tool to study certain forest-insect problems. Gosswald (1934) showed that the size of the frass pellets bore a constant relationship to the development of larval instars. Schwerdtfeger (1930) used frass drop in different types of stands to compare the effects on populations of *Bupalus piniaris* of airplane dusting, natural control factors, and food shortage due to overfeeding.

As no information was available regarding frass patterns for field-crop insects, such data had to be determined before further work could be undertaken on the correlation of frass measurements with environmental factors. This paper reports the results of frass studies of the armyworm which were undertaken in 1956 as a part-time program of investigation for Eastern Canada by the Division of Entomology, Crop Insect Unit, Dominion Department of Agriculture.

MATERIALS AND METHODS

Eggs were obtained from gravid females caught in a light trap and placed in rearing cages in the insectary at Fredericton, N. B. Fifty larvae at a time, hatching from these eggs, were placed in individual jelly jars and were reared separately. A small wad of cotton was first placed in each jar and covered with a tight-fitting disk of blotting paper. The frass samples were collected every 24 hours, and were immediately counted and measured. All frass measurements were made with an ocular micrometer disk in one eyepiece of a binocular microscope, using a 1.0X objective and a 12.5X ocular. Calibration with a Cenco micrometer stage scale showed, at this magnification, 40 ocular micrometer units were equal to 2 mm.

Treatment of Frass.—The larva in each jelly jar was removed every 24 hours. The blotting paper disk, carrying the frass, was then removed carefully, and the cotton and the jar were examined for any frass particles that might have fallen there. The cotton, with a drop of water added, was then replaced in the jar and a new blotting-paper disk was put in place, and the larva was returned to the jar and provided with fresh foliage. The disk that had been removed was then examined for frass pellets, which were counted and measured and were then stored in small pill boxes.

RESULTS AND DISCUSSION

Description of Frass.—The frass of first-instar *P. unipuncta* is very fine. The pellets of the later instars are coarse, and appear to consist of large particles of fibers promiscuously bound together; they are cylindrical in form and cut off obliquely at the ends (fig. 1). Frass is light green in color when newly ejected, but becomes dark brown after exposure to the air.

Weight of Frass.—For the first five instars there is very little difference between the wet and the dry weight of frass pellets, but these weights differ greatly in pellets of sixth-instar larvae. This is shown in figure 2, which is based on pellets of five males and five females reared at a mean temperature of 65° F.

Results of Leaching.—The frass, though composed largely of cellulose roughage, contains some soluble salts which are leached out during a rain. This causes the pellets to become lighter, but, due to the large roughage content, there is little change in their size and shape. To determine the loss of weight due to leaching, frass from larvae of the third to the sixth instars, inclusive, was exposed to various amounts of rainfall.

These larvae had been reared in the greenhouse, and their frass thus had not been exposed previously to moisture; it had been air-dried, and the weight and volume of the individual samples had been determined. After exposure, these samples were dried again and their weights and volumes were then redetermined. Rainfall readings were made from the Tipping Bucket Alter Rain Gauge at the Fredericton Research Station, and were checked with the Tipping Bucket Shielded Rain Gauge, which recorded 0.05 inches more than the former during the same period.

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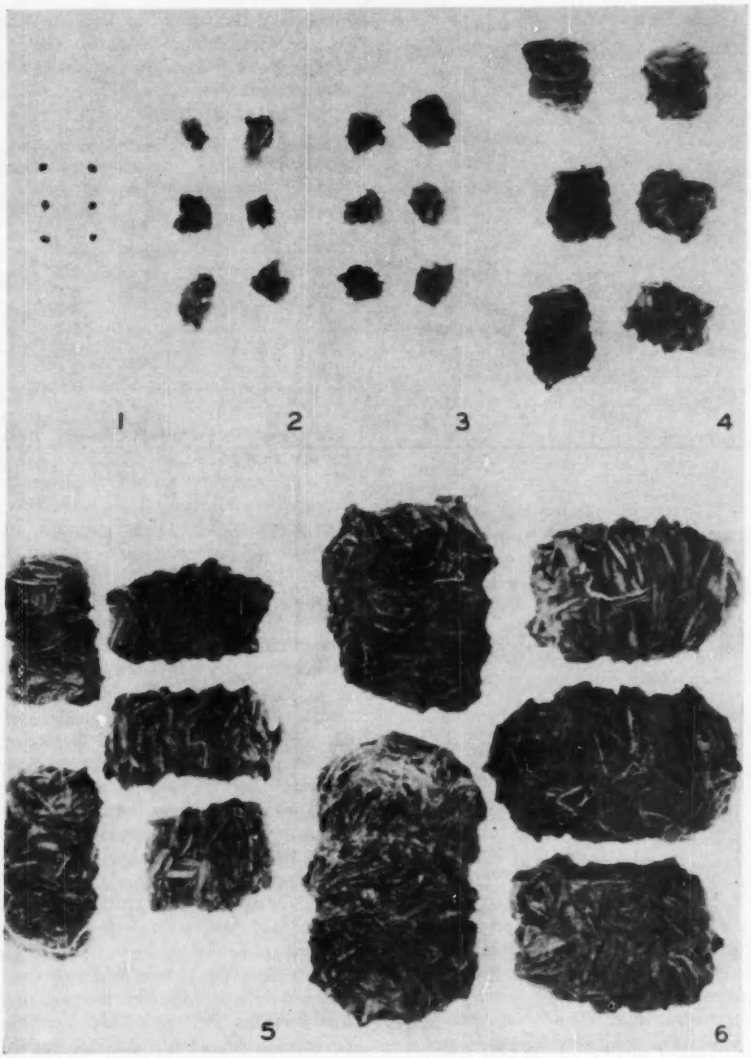

FIG. 1.—Frass pellets from each instar of *Pseudaletia unipuncta*, reared at a mean temperature of 70° F. Approximate magnifications: 1, x71; 2, x113; 3, x85; 4, x91; 5, x110; 6, x120.

Table 1 shows the amount of rainfall to which frass from each of the instars was subjected, and the resulting loss in weight.

These data agree more closely with those of Morris (1949) on the European spruce sawfly, *Gilpinia hercyniae* (Htg.), than with those of Thomson (1949) on the hemlock looper, *Lambdina fiscellaria lugubrosa* Hlst. Morris found that there was a loss of about 3% after exposure of frass to dew for several nights, which increased to 20% with exposure to 0.25 inch of rain and reached a maximum of 25% with larger amounts. Thomson found that the loss was 29.5% and 29.3% for frass from fourth- and from sixth-instar larvae, respectively, exposed to 0.8 inch of rain.

Frass Measurements.—Morris (1949) showed that pellet measurements could be used to advantage in determining larval instars in the field. Eckstein (1937), in his work on the nun moth, *Lymantria monacha* (L.), concluded that pellet size bears a more constant relation to instars than do measurements of the head capsule. The present study showed that instars could be determined readily from frass of larvae reared at an average mean temperature of

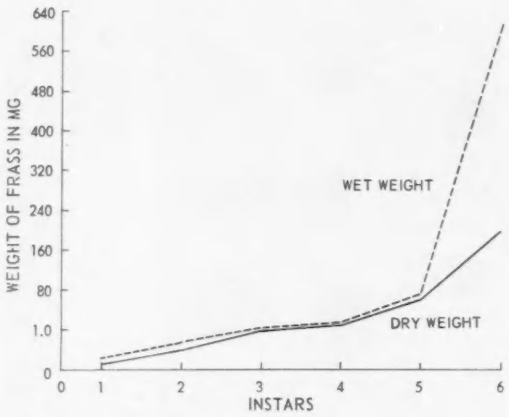

FIG. 2.—Average weight frass from respective instars of *P. unipuncta.*

67.6° F. and 75.2% average relative humidity; this was true for larvae reared within the normal range of temperature (65° to 75°), provided such factors as starvation or overcrowding were not introduced.

Table 1.—Percent of weight lost through leaching from frass of instars 3 to 6 of *Pseudaletia unipuncta.*

Rainfall, inches	Instar			
	3	4	5	6
0.01	2.0	5.8	11.8	6.4
0.27	—	—	21.2	24.5
0.53	8.6	14.6	29.2	25.8
1.07	—	—	35.7	30.4

Table 2 shows the weighted mean averages for pellet lengths and widths of the various instars of *P. unipuncta.* This progression is approximately geometric, with an average ratio of 1.8 between each instar and the preceding one. There was no significant difference between males and females in dimensions of the pellets, and results obtained from the two sexes are combined in this table.

Frass Yield.—The yield of frass from the various instars was recorded for rearings conducted at several combinations of temperature and humidities. These data are shown in table 3.

Table 2.—Weighted mean averages of frass-pellet lengths and widths (combined for males and females).

Instar	Length (mm.)	Width (mm.)
1	0.21	0.15
2	0.45	0.28
3	0.82	0.54
4	1.43	0.93
5	2.53	1.60
6	3.45	2.49

Though the total number of frass pellets produced during the whole larval period—whether for six or seven instars—was fairly constant, there was great variation at different temperatures and humidities in the numbers produced in the individual instars. Similarly, there were great variations in the total weights of frass produced and in the weights of the pupae that developed from the larvae. Increase in the weight of the frass was generally in accord with the greater number of pellets produced in the final instars, when the size of the pellets was considerably larger; but this increase in the weight of the frass was not correlated with a corresponding increase in the weight of the pupae. Rather, the reverse was true.

Figure 3 shows the average number of pellets excreted every 24 hours by 11 males and 6 females, together with the mean daily temperature and humidity for the period. In this experiment the larvae were reared on grain foliage. At the time of molting there was a period of from 12 to 48 hours, depending on the size of the larvae, when no frass was excreted. This accounts for the sharp drops on the graph, and clearly marks the end of each instar.

Analysis of Data.—In the analysis, the frass

Table 3.—Average numbers of frass pellets per instar, and average mean weights of frass drop and of pupae, from larvae reared at various temperatures and humidities. Fredericton, N. B., 1957.

Mean average Temp. (°F.)	R.H. (%)	Larvae No.	Sex	Average number of frass pellets: Instars 1	2	3	4	5	6	7	Total	Average weights: Air-dried pellets mg.	Pupae mg.
65.0	70.3	25	♂	122	132	129	82	48	125	x	638	299.6	410.0
65.0	70.3	25	♀	111	133	129	77	53	131	x	634	304.5	395.0
70.7	67.0	33	♂	70	79	55	104	111	156	x	575	493.3[b]	320.0
70.7	67.0	56	♀	72	84	72	102	106	174	x	610		310.0
70.7	67.0	3[a]	♂	38	45	94	43	67	92	151	530	400.7	280.6
70.7	67.0	11[a]	♀	42	34	57	66	81	95	181	556	456.8	282.8
62.7	90.7	7	♂	36	42	78	110	126	95	121	608	401.5	316.2
62.7	90.7	7	♀	32	40	69	114	121	90	134	600	350.9	289.3

[a] These larvae alternately fed and starved in successive 24-hour periods.
x = No seventh molt occurred under these rearing conditions.
[b] Combined average for both males and females.

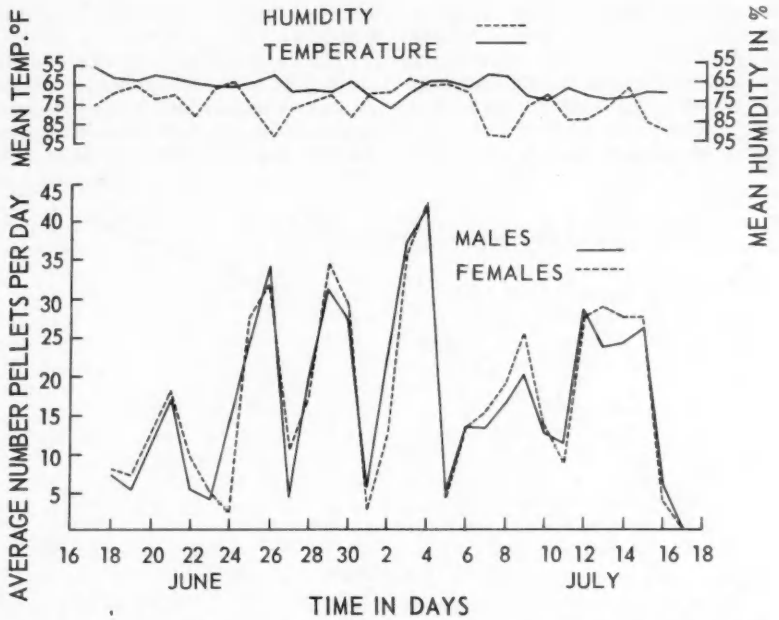

FIG. 3.—Average daily frass drop of insectary-reared *P. unipuncta*, with mean temperature and humidity for period.

measurements were considered the independent variable (X) and the development in days as the dependent one (Y), because the latter depended on temperature, humidity, etc., as well as food.

To determine whether there was a constant linear relationship between frass measurements and development, simple regression analyses were first tried for each instar. These showed that a high degree of correlation existed in the first to the fourth instars, inclusive, but not in the fifth and sixth. The type of analysis finally adopted was that of the second-degree curve for nonlinear correlation. This gave a significantly better fit than the straight line because the rate of increase of frass size declined as larval development progressed.

Table 4 summarizes the regression analyses for length and for width, respectively, of frass pellets, based on combined data for the two sexes. As the pooled regression analysis is valid, the separate analyses for males and females are not given. To facilitate their interpretation, the data are shown in graphic form in figures 4 and 5. These, like the tabular data, show that a definite relationship exists between development in days and both frass length and frass width.

Frass Drop and Food Consumed.—The relationship between frass drop and foliage consumption probably is of little value in studying the armyworm, but it could give an insight into the reason for its sudden appearance overnight in such numbers as to wipe out a grain field.

Table 4.—Summaries of regression analyses of frass length and width of males and females with development in days.

Source	DF	Sum of squares	Mean square	F	P
		Frass length			
Linear	1	6,599.40	6,559.40	970.5	<0.001
Quadratic	1	85.74	85.74	12.61	<0.001
Error	27	183.7	6.80		
Total	29	6,868.8			
Correction	1	21,081.2			
			$\rho = 0.986$		
			$\sigma = 1.76$		
		Frass width			
Linear	1	3,710.38	3,710.38	1,427.1	<0.001
Quadratic	1	95.74	95.74	36.8	<0.001
Error	29	75.4	2.60		
Total	31	3,881.5			
Correction	1	12,012.5			
			$\rho = 0.990$		
			$\sigma = 1.58$		

Frass drop has been shown by Morris (1949) and others to be closely correlated to food consumption, with respect to both absolute and relative rates. Gornitz (1933) demonstrated that both rates were influenced by temperature.

This correlation was examined in the present study, and table 5 gives the data obtained. These support this relationship. Pellet numbers were independent of foliage consumption, as was also shown by Morris (1949).

Davis and Satterthwait (1916), in their studies of the armyworm, showed that the durations of the first five instars did not differ greatly although the amount of foliage eaten per instar gradually increased from 0.03 square inch to 5 square inches. They found that the total food consumed during the larval period was 41.394 square inches, and that 34.128 square inches of this was consumed during the sixth instar alone.

This remarkable voracity of the armyworm in its last larval instar accounts for its sudden recognition in such enormous and destructive numbers. Such large consumption of food in the last instar is also common to a number of forest insects (Morris 1949).

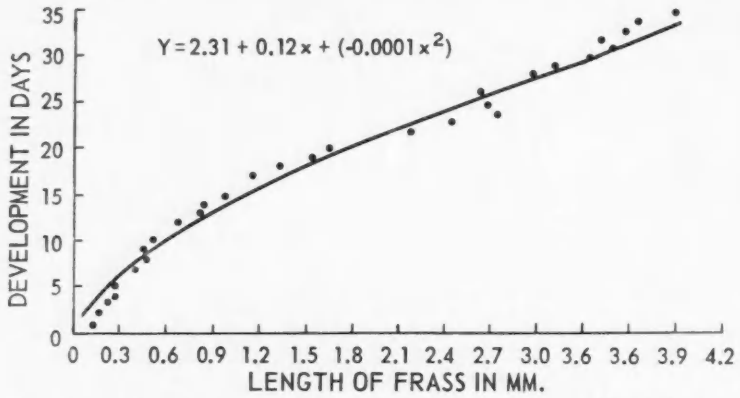

FIG. 4.—Relation of length of frass of *P. unipuncta* (males and females) to development in days.

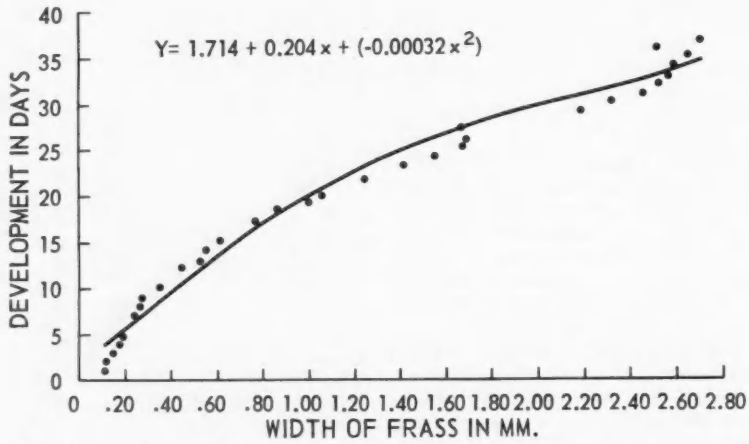

FIG. 5.—Relation of width of frass of *P. unipuncta* (males and females) to development in days.

Table 5.—Relation between length of development, foliage consumption, and frass yield by instars of the armyworm, *Pseudaletia unipuncta*.

Instar	Development		Foliage consumed		Frass ejected		Pellets ejected	
	days	%	*gm.*	%	*gm.*	%	*No.*	%
1	4.4	13.33	2.5	0.07	0.1	<0.01	66.1	11.36
2	4.2	12.73	7.0	0.19	0.35	0.115	129.5	22.25
3	4.1	12.42	32.9	0.87	2.06	0.68	122.4	21.03
4	4.3	13.03	112.8	2.99	10.59	3.48	76.1	13.07
5	5.6	16.97	501.4	13.31	61.96	20.34	54.1	9.30
6	10.4	31.52	3,110.9	82.57	229.64	75.37	133.8	22.99
Total	33.0	100.00	3,767.5	100.00	304.70	99.99	582.0	100.00

Monro (1935) and others have presented data on feeding rates of other insects which were merely the weight of food consumed in a given period divided by the weight of frass. This is not practical with the armyworm, as it is a very wasteful feeder. Throughout most of the first instar the larvae only skeletonize the leaves; later, just before molting, they begin to eat the entire leaf surface. In the field, the larvae feed on the undergrowth, such as clover, in the grain fields and, later, on the lower leaves of the grain itself. They are not very active in the first five

instars, and remain in one place if food is available. In the sixth instar, however, they move about continually and feed voraciously. The relative amount of injury in the field—particularly with small grains such as oats and barley—is much greater than the actual amount of food consumed. The worms clip off the grain heads, then travel down the stalks, leaving them uneaten, and move on to new ones. This drive of armyworm larvae has the effect of uniting them. They enter a grain field and practically clean it, leaving the ground whitened with uneaten grain heads as they march in droves across roads and fields to other grain patches where they repeat their destructive work.

The average weight of green foliage consumed per larva is approximately 3,767 grams. The average pupal weight, based on laboratory rearings, is approximately 388 grams. During larval life, therefore, the armyworm consumes about 9.7 times its own pupal weight in food. The corresponding factors for *Porthetria dispar* and for *Gilpinia hercyniae* have been reported as 8.8 and 13.6, respectively.

FACTORS INFLUENCING FRASS YIELD

Frass yield is dependent on a number of factors, among which are larval development, temperature, available food supply, and others. All such factors must be considered in any attempt to correlate frass yield with development.

Larval Development.—One experiment performed in the present study was conducted to determine what effect, if any, the availability of food supply had on development. Twenty-five larvae were reared individually in jelly jars. Throughout their larval period they were alternately provided with food for 24 hours, then deprived of all food for a similar period. Sixty percent of the females in this experiment, and fifty percent of the males, molted seven times, while all of the larvae molted six times in the control experiment where they were fed continuously. The data in table 6 show that in comparison with the controls the total larval span of the females fed only on alternate days was lengthened by 68% and that of the males by 29%. Also, 71% of those that completed larval development were females, as compared with 50% in the controls. Thus the availability of food seemed not only to affect the length of larval development, but also to have a differential effect upon the sexes. The length of the pupal period, however, remained practically constant. The total number of frass pellets produced did not increase proportionally with the increase in the number of days spent in the larval stage.

Table 6.—Comparison of development and frass-pellet production of armyworm larvae deprived of food during alternate 24-hour periods and other larvae fed continuously.

No. of larvae	Pupated		Average no. of days		Average no. of pellets produced
	Number	Sex	Larval	Pupal	
25[a]	15	♀	53.8	14.7	528.0
	6	♂	45.3	16.3	542.8
5[b]	2	♀	32.0	15.5	484.0
	2	♂	35.0	18.5	509.5

[a] Deprived of food during alternate 24-hour periods.
[b] Fed continuously.

Temperature.—Gornitz (1933) showed that pellet yield was a more sensitive tool than weight of frass, and used pellet counts in studying the effects of meteorological factors. Morris (1949) found temperature very important in regard to both insect activity and development. In the present study an experiment was conducted to determine the relation, if any, between temperature and frass drop and frass size.

Fifty larvae were reared individually in jelly jars in the insectary, where the average mean temperature for the period was 72.6° F. Twenty larvae of another series were reared in an incubator at an average mean temperature of 62.7°. The results, presented in table 7, indicated a definite relationship between temperature and both pellet yield and pellet size. The frass pellets of all instars were longer at the higher temperature, but the width of the pellets was not similarly affected until the fourth instar was reached. On the other hand, the number of days, the number of pellets produced, and the weight of the pellets were greater at the lower temperature, and the average weight of the pupae produced was less than at the higher one. The sex ratio was approximately 50:50 at each temperature.

Table 7.—Duration of the larval instars of *Pseudaletia unipuncta*, and size and number of frass pellets produced, when reared in two different temperature ranges. Fredericton, N. B., 1957.

Instar	Temp.[a]	Average number		Range of pellet size		Average weight of	
		Days	Pellets	Length (mm.)	Width (mm.)	Total frass (mg.)	Pupae (mg.)
1	72.6	5	66.1	0.13–0.27	0.11–0.19		
1	62.7	14.8	35.5	0.11–0.20	0.11–0.17		
2	72.6	4	129.5	0.40–0.53	0.25–0.35		
2	62.7	6.2	41.5	0.11–0.31	0.23–0.35		
3	72.6	4	122.4	0.67–0.97	0.45–0.61		
3	62.7	5.7	77.8	0.41–0.52	0.45–0.66		
4	72.6	5	76.1	1.16–1.58	0.77–1.25		
4	62.7	6.7	110.3	0.88–1.40	0.63–1.00		
5	72.6	6	54.1	2.17–2.64	1.41–1.50		
5	62.7	7	117.3	1.40–1.90	0.99–1.46		
6	72.6	9	133.8	2.97–3.87	2.18–2.69		
6	62.7	15.1	219.5	1.78–2.58	1.47–1.96		
Totals	72.6	33.0	582.0			294.6	388.0
	62.7	55.1	601.9			367.6	311.7

[a] Average of mean daily temperatures for the period.

Parasitism.—Parasitism of the armyworm during and after the heavy outbreak remained at an extremely low level, and therefore it was not taken into account in this study. Tower (1916) showed that parasitized larvae of *P. unipuncta* consumed approxi-

mately half as much foliage as did healthy larvae, and noted that parasitism could be detected in the third and fourth instars by a decrease both in the amount of food eaten and in the amount of frass produced. Some parasites killed the larvae in the fifth instar, while others which allowed the armyworm to pupate emerged from the pupal case.

Morris (1949) stated that Bird had used the number of pellets dropped after the incubation period to determine the relative pathogenicities of various disease extracts. Parasites could surely be studied in the same way, and the effectiveness of the various parasitic species could readily be shown.

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SCIENTIFIC NOTES

The Estimation and Extraction of Acetylcholine from Nervous Tissue of the American Cockroach[1]

E. H. COLHOUN AND J. GILLEBERG

Pesticide Research Institute, Canada Department of Agriculture, London, Ontario

Although the nervous tissue of cockroaches contains higher titres of acetylcholine (ACh) than vertebrate nervous tissue on a wet-weight basis, it is necessary to use the nerve cords of a number of cockroaches for the assay of ACh by use of the rectus abdominis muscle of the frog. The number of nerve cords needed may be reduced by the elimination of any factor that tends to destroy ACh during the extraction procedure, or any that might reduce the threshold sensitivity of the muscle preparation to ACh. The results reported here show that trichloroacetic acid (TCA), when used in the method of Lewis and Smallman (1956) and Lewis and Fowler (1958) for the extraction of ACh from insect tissue, has a depressant effect on the rectus abdominis muscle of *Rana pipiens*.

The thoracic nerve cord of the cockroach *Periplaneta americana* L. was used for all experiments, as this tissue has a high ACh content (Colhoun 1958). Five to ten thoracic nerve cords, dissected by the method described by Colhoun (loc. cit.), were pooled and placed for 30 seconds in 5 ml. of boiling frog Ringer's solution, pH 3.9 ($NaHCO_3$ buffer omitted). Five drops of Gramercy Universal Indicator were added to the Ringer's solution so that approximate pH changes could be observed. When cool, the nerve cords were homogenized in the same Ringer's solution in the following manners: (1) without acid; (2) 1 percent final concentration of TCA; (3) 0.5 percent final concentration of TCA; (4) 2 N HCl to give pH 1.5, equivalent to that found with TCA. The homogenates were centrifuged for 3 minutes at 2,000 g. The supernatants were decanted and the pH was adjusted to 6.0 by the addition of 0.3 N NaOH, with constant stirring. Unbuffered Ringer's solution was added to give a final volume of 10 ml. This was divided into two 5-ml. aliquots. One of these was brought to pH 11.5 by the addition of 2 N NaOH, to hydrolyze ACh; then, after 15 minutes, it was brought to pH 6.0 with 2 N HCl. This aliquot was termed the blank. ACh was added to it during the assay procedure, and in this way correction was made for possible sensitizing agents. The unhydrolyzed aliquot was termed the extract.

Assays for ACh were carried out according to the method of Colhoun (1958). Amounts of extract up to 1 ml. were combined with $NaHCO_3$-buffered, eserinized Ringer's solution, final volume 5 ml., and placed in the muscle bath. The muscle was allowed to contract for 1½ minutes; the bath was then flushed several times with Ringer's solution and the muscle rested for 1½ minutes. The height of contraction obtained with the extract was matched with a known amount of ACh contained in an equal volume of blank and Ringer's solution.

The depressant effect of TCA on the contraction of the rectus abdominis muscle is shown in figure 1, c, d. The muscle contraction was not depressed by extracts containing HCl (b), or by extracts which contained neither HCl or TCA. A TCA concentration of 0.5 percent in the initial extract resulted in an immediate depression of the muscle (c). After the application of extract containing 1 percent TCA in the original volume, the height of the contraction was reduced by about 50 percent (d). The variability of the effect with TCA is shown in figure 2 (b), where the reduction in muscle contraction is not quite so pronounced but is still apparent. Muscles obtained from frogs in early spring were

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particularly sensitive to TCA. When aliquots of extracts containing TCA were applied to a muscle at the beginning of an experiment, the depressant effect could not be reversed by repeated washings with Ringer's solution. The muscle remained depressed even when nonacid extracts were then assayed on the muscle.

In spite of the depressant effect, the presence of TCA did not affect the result of the assay. Thus the estimates of ACh (μg. per ml. of extract) obtained were (1) no acid, 0.32; (2) HCl, 0.30; (3) 1 percent TCA, 0.30; (4) 0.5 percent TCA, 0.30. The use of TCA as a protein denaturant is questionable, for in these experiments the nervous tissue was first boiled before homogenization. The use of TCA necessitates the addition of NaOH to bring the pH of the extract from 1.5 to 6.0. This is an additional hazard, for ACh can be hydrolyzed unless the extract is stirred vigorously during the addition of the alkali.

These results indicate that the presence of TCA in extracts of nervous tissue of the cockroach has a depressant effect on the rectus abdominis muscle of the frog. The results also show that the ACh of the thoracic nerve cord of the cockroach can be extracted satisfactorily without the use of TCA. The suggested procedure for extraction of ACh from small amounts of insect neural tissue is as follows: place the tissue in an aliquot of boiling, frog Ringer's solution, pH 3.9, for 30 seconds; cool, homogenize, centrifuge, decant the supernatant, and divide into two aliquots; treat one aliquot with 2 N NaOH to destroy the ACh, then neutralize with 2 N HCl. If large amounts of the undestroyed extract are used for the bioassay procedure, the pH can be adjusted to neutrality by the use of weak NaOH or $NaHCO_3$.

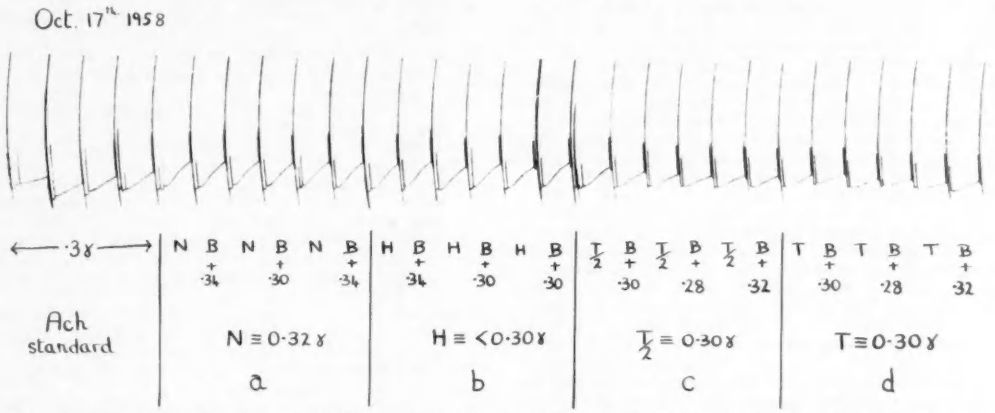

FIG. 1.—The depressant effect of TCA-extracted ACh upon the rectus abdominis muscle of the frog. ACh extracted by (a) boiling, (b) boiling and 2 *N* HCl, (c) boiling and 0.5 percent TCA, and (d) boiling and 1.0 percent TCA.

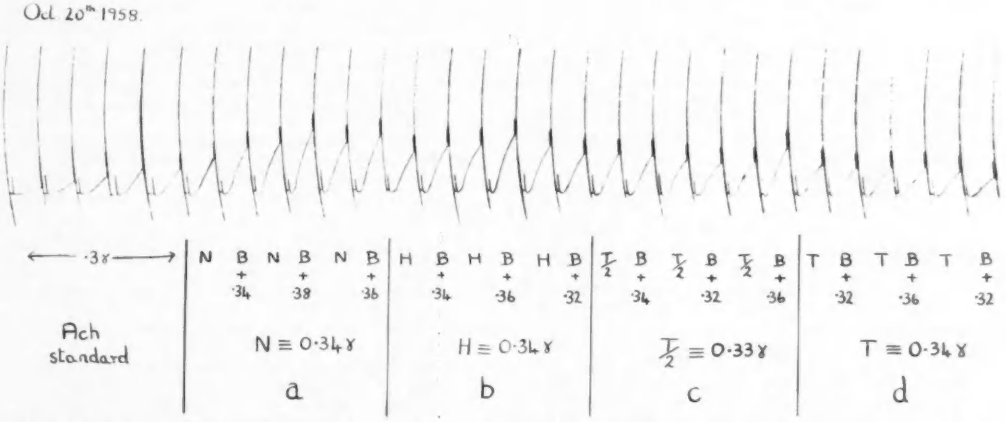

FIG. 2. The variability in the height of muscle contraction with 1.0 ml. of extract when compared to figure 1 and the effect of TCA. ACh extracted by (a) boiling, (b) boiling and 2 *N* HCl, (c) boiling and 0.5 percent TCA, and (d) boiling and 1.0 percent TCA.

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Baja California Triatominae (Hemiptera: Reduviidae) and their Hosts (Rodentia: Cricetidae)[1]

RAYMOND E. RYCKMAN[2] AND ALBERT E. RYCKMAN[3]

The distribution, taxonomy, and ecology of Triatominae in Baja California has not been well understood This primarily is due to the relative inaccessibility of the peninsula by surface transportation; secondarily, because the collection of these bugs usually requires a pick and shovel rather than an insect net. Most of the collections reported here were made on a memorable collecting trip by Jeep truck to the southernmost tip of the peninsula, in 1957. These collections were made possible by a grant from the Associates in Tropical Biogeography of the University of California at Berkeley, with additional funds from the College of Medical Evangelists of Loma Linda. All *Neotoma* were identified by mammalogists at the U. S. National Museum.

Prior to this report, the genus *Paratriatoma* was not known to occur in the Republic of Mexico; however, on ecological grounds one would suspect that this genus should have occurred there. The only representative of the *Triatoma protracta* group known to be present on the peninsula was the morphologically small "subspecies" *peninsularis*. On the basis of genetic evidence which will be published in another report, *peninsularis* is treated here as a full species; hence, *Triatoma protracta* (Uhler) was not previously known from Baja California. One would have expected this species to have occurred south of the border because of its close proximity to Baja California at San Diego, California. *Triatoma rubida rubida* (Uhler) was described from Cabo San Lucas, at the tip of the peninsula, but the extent of its distribution northward was not known. The genus *Dipetalogaster* has previously been taken in the southern part of Baja California, Territorio Sur. We are not able to add further information on the distribution or ecology of this genus.

Triatominae collections, hosts, and plant associates are given in the lists which follow. Unless otherwise stated, all collections were made by Raymond E. Ryckman, Albert E. Ryckman, Joseph V. Ryckman, and Dean Spencer.

Paratriatoma hirsuta Barber

30 mi. N.W. San Augustin, June 27, 1957; elevation 2000 ft. Host: *Neotoma lepida.*

25 mi. S. E. San Augustin, June 28, 1957; elevation 2500 ft. Host: *Neotoma.* One adult female; one fifth-instar, one third-instar nymph.

25 mi. N. Punta Prieta, June 30, 1957; elevation 2400 ft. Host: *Neotoma.* Habitat, in *Yucca valida.* Three fifth-instar nymphs.

12 mi. N. Punta Prieta, July 1, 1957; elevation 2000 ft. Host: *Neotoma.* Habitat, in *Machaerocereus gummosus.* Two adult males; two third-instar nymphs.

31 mi. S. Punta Prieta, July 2, 1957; elevation 1100 ft. Host: *Neotoma.* Habitat, nest built in low cliff. One fifth-instar nymph.

Jacumba, San Diego Co., California (taken near U.S.-Mexico border), July 28, 1955 (R. E. Ryckman, R. Walter). Host: *Neotoma fuscipes macrotis.* Habitat, in *Quercus, Juniperus, Rhus,* and *Yucca.*

This species has also been taken 7 mi. E. Julian, San Diego Co., California, October 8, 1953 (R. E. Ryckman, R. D. Lee, C. T. Ames, C. C. Lindt). Host: *Neotoma fuscipes macrotis.* Habitat, in *Quercus.*

Triatoma rubida (Uhler)

The type was taken from Cabo San Lucas, at the tip of the peninsula south of La Paz. Additional collections were made by E. S. Ross and R. A. Carillo near La Paz in 1940.

5 mi. E. San Ignacio, July 5, 1957; elevation 600 ft. Host: *Neotoma.* One adult male, one adult female.

3 mi. S. El Coyote Bay (W. Concepcion Bay), July 8, 1957; elevation 400 ft. Host: *Neotoma lepida molagrandis.* Habitat: in *Lophocereus schottii.* Seventeen second- to fourth-instar nymphs.

38 mi. N.E. Comondu, July 9, 1957; elevation 2000 ft. Host: *Neotoma.* Habitat, in *Machaerocereus gummosus.* Seven nymphs.

230 km. N. La Paz, or 28 mi. S. Santo Domingo, July 10, 1957; elevation near sea level. Host: *Neotoma lepida molagrandis.* Habitat, in *Prosopis* and *Opuntia* (cholla). Three adult males; eleven nymphs in first to third instars.

98 km. N.W. La Paz, July 11, 1957; elevation 900 ft. Host: *Neotoma* lodge, occupied by *Spilogale* skunk. Habitat, in *Machaerocereus gummosus.* Two adult males; one fifth-instar nymph.

5 mi. N. Todos Santos, July 14, 1957; elevatoin 800 ft. Host: *Neotoma.* Habitat, in unidentified thorn bush. One adult male, one adult female. (On two occasions active nests of the quail *Lophortyx californicus achrusterus* were found inside wood-rat lodges; a female quail was incubating six eggs within this rat lodge.)

2 mi. N.E. Cabo San Lucas, July 15, 1957; elevation 200-400 ft. Host: *Neotoma lepida arenacea.* Habitat, under boulders. One adult flew to camp light.

3 mi. N. Santiago, July 17, 1957; elevation 500 ft. One adult flew to camp light.

9 mi. N. E. Santiago, July 17, 1957; elevation 500 ft. Host: *Neotoma.* Habitat, in *Opuntia* (flat pad). One adult male.

Triatoma protracta (Uhler)

Rough-egg Form

7 mi. E. Rosario, June 26, 1957; elevation 400 ft. Host: *Neotoma lepida egressa.* Habitat, in *Machaerocereus gummosus.* One adult male, one adult female.

4 mi. W. Jacumba, San Diego Co., California (U.S.-Mexico border), July 28, 1955 (R. E. Ryckman, R. Walter). Host: *Neotoma fuscipes macrotis.* Habitat, in *Salix.*

Triatoma protracta (Uhler)

Smooth-egg Form

Santo Tomas, August 6, 1951; elevation near sea level (R. E. Ryckman, C. C. Lindt). Host: *Neotoma.* Habitat, in *Quercus.*

5 mi. S. San Quintin, November 4, 1953; elevation near sea level (R. E. Ryckman, C. P. Christianson, C. C. Lindt). Host: *Neotoma.*

N. Colonia Guerrero, November 3, 1953; elevation near sea level (Ryckman, Christianson, Lindt). Host: *Neotoma fuscipes martirensis.* Habitat, in *Agave shawii.*

San Jose (Mehling Ranch), November 6, 1953; elevation 2000 ft. (Ryckman. Christianson, Lindt). Host: *Neotoma fuscipes martirensis.* Habitat, in *Salix.*

Triatoma protracta (Uhler)

Egg Type Undetermined

Cedros Island, August 5, 1922 (C. D. Hanna).

Triatoma peninsularis Usinger

20 mi. N.W. La Paz, July 16, 1938 (A. E. Michelbacker, E. S. Ross). Collected at lights.

Venancio, July 17, 1938 (Michelbacker, Ross).

28 mi. S. Santo Domingo, July 10, 1957; elevation near sea level. Host: *Neotoma lepida molagrandis.* Plant associates: *Machaerocereus gummosus, Prosopis,* and *Opuntia* (cholla). One adult male; one third-instar nymph.

98 km. N.W. La Paz, July 11, 1957; elevation 900 ft. Host: *Neotoma.* Rat lodge occupied by *Spilogale* skunk. Plant associate, *Machaerocereus gummosus.* Seven adult males; 50 nymphs in second to fifth instars.

5 mi. N. Todos Santos, July 14, 1957; elevation 800 ft. Host: *Neotoma.* Plant associate, unidentified thorn

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[2] Department of Microbiology, The College of Medical Evangelists, Loma Linda, California.

[3] Redlands, California.

bush. One adult male, one adult female; 15 nymphs in second to fifth instars.

2 mi. N. E. Cabo San Lucas, July 15-16, 1957; elevation near sea level. Host: *Neotoma lepida arenacea* Allen. Habitat, in *Machaerocereus gummosus* and in large boulders. Two nymphs.

9 mi. N.E. Santiago, July 17, 1957; elevation 500 ft. Host: *Neotoma*. Plant associate, *Opuntia* (flat pad). Two adult males: 47 nymphs in first to fifth instars.

11 mi. S. Santa Rosalia, July 6, 1957; elevation near sea level. Host: *Neotoma*. Habitat, wood-rat nest in pile of stones and *Opuntia* (cholla). Two fifth-, two fourth-, two third-, two second-, and one first-instar nymphs.

Dipetalogaster maximus (Uhler)

Cabo San Lucas (southernmost tip of the peninsula; collected on the Albatross Expedition).

10 km. S.W. La Paz (R. A. Carillo) (en las canadas de El Cajoncito y Vinoramas).

DISCUSSION

Paratriatoma hirsuta has not previously been known from Mexico or from San Diego County, California, by published reports or museum specimens. This species was collected in five localities in Baja California and two localities in San Diego County; one collection was on the international boundary at Jacumba. The southernmost point where this species was collected lies approximately 37 miles north of the boundary between the State of Baja California and the Southern Territory of Baja California. The known range of this species has thus been extended 400 miles to the south, and 325 miles into Mexico.

Triatoma rubida rubida has previously been collected from the Cape at the tip of Baja California, and near La Paz, which also is in the southern portion of the peninsula. Ten collections were made throughout the Southern Territory; the most northern collection for this species was obtained 50 miles south of the border between the Southern Territory and the State of Baja California. This is a 275-mile northward extension of the known range of this species. *Triatoma rubida rubida* on the peninsula appears to be geographically isolated from *Triatoma rubida uhleri* in the Colorado Desert of Imperial County, California.

Triatoma peninsularis was collected 20 miles northwest of La Paz in 1938 by Michelbacker and Ross. Until the 1957 expedition this species was known only from the types, and no information was available on its biology. *T. peninsularis* was collected from its natural reservoir host at six sites which ranged from the northern Plains of Magdalena and south of Santa Rosalia to within 2 miles of the last projection of land at the Cape of San Lucas. Its known range has been extended 125 miles northward, and 90 miles southward. All eggs laid by these populations were of a rough type.

Triatoma protracta protracta (rough-egg population) has been collected from two Baja, California localities, one near Rosario, the other at the international boundary at Jacumba, San Diego County, California. The rough-egg population was not previously known to extend into Lower California. The smooth-egg population of *T. protracta* occurs in the coastal region of western San Diego County and the coastal belt of northwestern Baja California.

Dipetalogaster maximus has not been reported since the first two collections recorded by Mazzotti (1940) and Usinger (1944). The host for this species is not known, and probably is not a wood rat (*Neotoma*).

The hosts from which *Paratriatoma hirsuta* was collected were *Neotoma fuscipes macrotis* and *Neotoma lepida*, the latter in a zone of intergradation between subspecies *gilva* and *intermedia*. *Triatoma r. rubida* was collected in association with *Neotoma lepida molagrandis* and *N. l. arenacea*. The rough-egg population of *Triatoma p. protracta* was collected in the nests of *Neotoma lepida egressa* and *N. fuscipes macrotis*, the smooth-egg population from *N. fuscipes martirensis*. No host has been reported previously for *Triatoma peninsularis*, which was collected from nests of *Neotoma lepida molagrandis* and *N. l. arenacea*.

REFERENCES CITED

Mazzotti, L. 1940. Triatomideos de México y su infeccion natural por *Trypanosoma cruzi* Chagas. Rev. Med. Mexicana 20(358): 95-110.

Usinger, R. L. 1944. The Triatominae of North and Central America and the West Indies and their public health significance. U. S. Publ. Health Serv., Publ. Health Bull. 288. 83 pp.

BOOK REVIEW

A REVISIONAL STUDY OF THE BEES OF THE GENUS *PERDITA* (F. SMITH), WITH SPECIAL REFERENCE TO THE FAUNA OF THE PACIFIC COAST (HYMENOPTERA: APOIDEA). PARTS I-IV. P. H. TIMBERLAKE, University of California Publ. Ent. Vol. 9, No. 6, p. 345-432, pl. 13-26. 1954. $1.25. Vol. 11, No. 5, p. 247-350, pl. 27-41. 1956. $2.00. Vol. 14, No. 5, p. 303-410, pl. 4-15. 1958. $2.00. Vol. 17, No. 1, p. 1-156, pl. 1-17. 1960. $3.00.

This is a monumental study of the second largest genus of bees in North America (the largest is *Andrena*). According to the author, Part V is nearly ready for submission and Part VI is in progress. He estimates that between 200 and 300 species are still to be treated, not counting the many Mexican species that have never been collected.

Prior to this study the identification of most species of *Perdita* by anyone but Mr. Timberlake has been practically hopeless. This has not been caused by any intrinsic difficulty in separating the species, but rather to the large number of species, many of them undescribed, and the absence of revisional papers.

Part I begins with a key to the 18 subgenera considered. Each subgenus is then handled with a subgeneric description, a key to the species, and descriptions of new ones. In many cases previously described species are merely listed bibliographically along with distribution and flower records. However, previously undescribed sexes are described and complete descriptions are given for most Pacific coast species and a number of others for which type material or material reliably compared with the holotype was available. The larger subgenera are divided into species groups, some of which are keyed separately. Nearly all species are illustrated with line drawings of dorsal and lateral aspects of the male genitalia and ventral aspects of the subgenital plates.

The succeeding parts follow the same format. Appendices in each of these supply supplementary information for subgenera treated in the previous parts. In all, Parts I through IV treat 305 species in 19 subgenera, although synonymy in the later parts reduces the number of species somewhat.

The keys were found to be generally workable and to place a minimum of reliance on variable or hidden characters. A key to the species groups of the huge subgenus *Perdita* would be helpful.

This work, which is the culmination of many years of intensive study, makes available for general biological study a large group of organisms heretofore cloaked in obscurity. *Perdita*, composed as it is of oligolectic species, should be an ideal subject for fundamental studies on insect-flower relationships.

G. E. BOHART
Entomology Research Division
U. S. Department of Agriculture

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